Diversité, biogéographie et écologie des Collodaires (Radiolaires) dans l'océan mondial

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A mes grands-parents, Claude et Pierre…

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Abstract

Collodaria (Radiolaria) are unicellular marine eukaryotes (protists) belonging to the super-group Rhizaria. Collodarian species contribute to planktonic communities as large solitary cells or can form large gelatinous colonies. They are heterotrophic organisms feeding on other plankton, which also systematically harbour intracellular symbiotic microalgae. Recent environmental molecular diversity surveys demonstrated their important contribution to planktonic communities and their worldwide occurrence in the global ocean. However, knowledge on their diversity, biogeography and ecology is paradoxically very poor.

In the first part of this thesis I performed detailed morphological analyses (electron and optical microscopy) combined with a molecular phylogeny based on the 18S and 28S rRNA genes, sequencing for a total of 75 distinct colonial and solitary specimens. Ultimately, this work led to the revision of the Collodaria classification and to the construction of a robust morpho-molecular reference database. Then, this morpho-molecular framework allowed the exploration of Collodaria biodiversity through a metabarcoding approach across samples collected in the global ocean during the *Tara* Ocean expedition. The cosmopolitan distribution of the different collodarian taxa in the surface oceans revealed a higher biodiversity in the vast oligotrophic inter-tropical open oceans. Collosphaeridae were predominantly found in the open oceans while the Sphaerozoidae were the dominant family in the less diverse coastal regions. The newly defined Collophidiidae were rarely encountered in the photic zones at all latitudes, suggesting that they inhabit a different ecological niche. Finally, I also used the *in situ* imaging system Underwater Vision Profiler (UVP5) to quantitatively explore the abundances and biomasses of collodarian and rhizarian in the global ocean. This approach revealed that the Rhizaria were a major component of the meso- and macro-plankton, constituting up to 4.5% of the global carbon standing stock in the upper 200 m of the world oceans. More specifically, Collodaria were the most important rhizarian groups in the first 100 m of the oceans, and their distribution suggested that photosymbiosis might be an important factor explaining their success in oligotrophic regions where they are particularly abundant. Besides the improvement of our knowledge on the diversity, biogeography and ecology of Collodaria in the global ocean, this thesis highlights the relevance to combine and/or use alternative sampling and analytical procedures such as high-throughput sequencing and *in situ* imaging technologies to study marine protists in their environment.

Keywords: Collodaria; Radiolaria; Rhizaria; Global ocean; *In situ* imaging; High-throughput sequencing; Metabarcoding; Integrative taxonomy; Molecular phylogeny; Zooplankton.

Résumé

Les Collodaires (Radiolaires) sont des eucaryotes unicellulaires (protistes) marins appartenant au super-groupe des Rhizaria. Tandis que certains sont caractérisés par un mode de vie colonial, d'autres sont observés sous la forme de larges organismes solitaires. Les Collodaires sont des protistes hétérotrophes, prédateurs de plancton, mais également hôtes systématiques de micro-algues photosynthétiques intracellulaires. Les récentes analyses de leur diversité moléculaire dans l'environnement ont démontré leur importante contribution aux communautés planctoniques ainsi que leur distribution globale dans l'océan mondial. Cependant, nos connaissances sur leur diversité, biogéographie et écologie restent paradoxalement parcellaires.

La première partie de cette thèse a été dédiée à des études morphologiques détaillées (en microscopie électronique et optique) et combinées à une phylogénie moléculaire élaborée en séquençant les sous-unités 18S et 28S de l'ADN ribosomal pour 75 spécimens, coloniaux ou solitaires. Ce travail a abouti à la réévaluation de la classification des Collodaires et à l'élaboration d'une base de référence morpho-moléculaire robuste. Par la suite, ce cadre de référence morpho-moléculaire a permis d'explorer la biodiversité des Collodaires grâce à une approche de *metabarcoding* appliquée à une série d'échantillons collectés dans l'océan mondial pendant l'Expédition *Tara* Océans. La distribution cosmopolite à la surface des océans des différents taxons qui composent les Collodaires, a révélé une diversité plus importante dans les vastes régions océaniques intertropicales et oligotrophiques. Les Collosphaeridae ont été principalement observés en pleine mer alors que les Sphaerozoidae formaient la famille dominante dans les régions côtières, où la biodiversité des Collodaires était plus faible. Les Collophidiidae, formellement décrits au cours de thèse, ont rarement été rencontrés dans les zones photiques, quelque que soit la latitude, suggérant ainsi qu'ils occupent une niche écologique particulière. Enfin, j'ai également employé la technologie d'imagerie *in situ* Underwater Vision Profiler (UVP5) afin d'explorer de façon quantitative les abondances et biomasses des Collodaires et des Rhizaria, à travers l'océan mondial. Cette approche a révélé que les Rhizaria forment un composant majeur du méso- et macro-plancton, et représentent jusqu'à 4,5% de la biomasse globale des 200 premiers mètres de l'océan mondial. Plus particulièrement dans les 100 premiers mètres, les Collodaires constituent le groupe le plus important des Rhizaria et leur distribution suggère que la photosymbiose pourrait influencer leur succès dans les régions oligotrophiques où ils sont particulièrement abondants. Au-delà d'améliorer notre compréhension de la diversité, la biogéographie et l'écologie des Collodaires dans l'océan mondial, ce travail de thèse souligne la pertinence de combiner et d'utiliser des approches alternatives d'échantillonnage et d'analyses tel que le séquençage haut-débit et l'imagerie *in situ* dans l'étude des protistes marins dans leur environnement.

Mot Clés : Collodaires ; Radiolaires ; Rhizaria ; Océan mondial ; Imagerie *in situ* ; Séquençage haut-débit ; *Metabarcoding* ; Taxonomie intégrative ; Phylogénie moléculaire ; Zooplancton.

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INTRODUCTION GENERALE

« There you can do much and as soon as you have entered into this pelagic wonderland you will see that you cannot leave it. »

Johannes Müller to Ernst Haeckel (1854)

1. Plancton et écosystèmes océaniques

1.1. Le Plancton au cœur des écosystèmes marins

Loin de l'image d'une étendue désertique où la vie ne s'observe qu'à travers de larges organismes, les océans, couvrant environ 70% de la surface terrestre, constituent le plus grand écosystème de la Terre où fourmille une infinité de formes de vie différentes (Angel, 1993). Moins connu que les grands mammifères marins qui peuplent les océans, mais qui ne représentent qu'une part infime de la biomasse marine (Sheldon et al., 1972), le plancton, collection éclectique réunissant virus, bactéries, organismes unicellulaires eucaryotes (aussi appelés « *protistes* ») ou pluricellulaires, dérivant au gré des courants, constitue le cœur même de la vie marine. À la fin des années 1970, le plancton fut catégorisé en plusieurs classes de tailles (Figure 1), du pico- au méga-plancton, couvrant plusieurs ordres de grandeurs (Sieburth, 1979). Dans cette vaste gamme de tailles, le plancton marin rassemble une multitude d'organismes, certains photosynthétiques, d'autres hétérotrophes et même certains mixotrophes, qui combinent une activité autotrophique et hétérotrophique (Caron et al., 2012 ; Flynn et al., 2013).

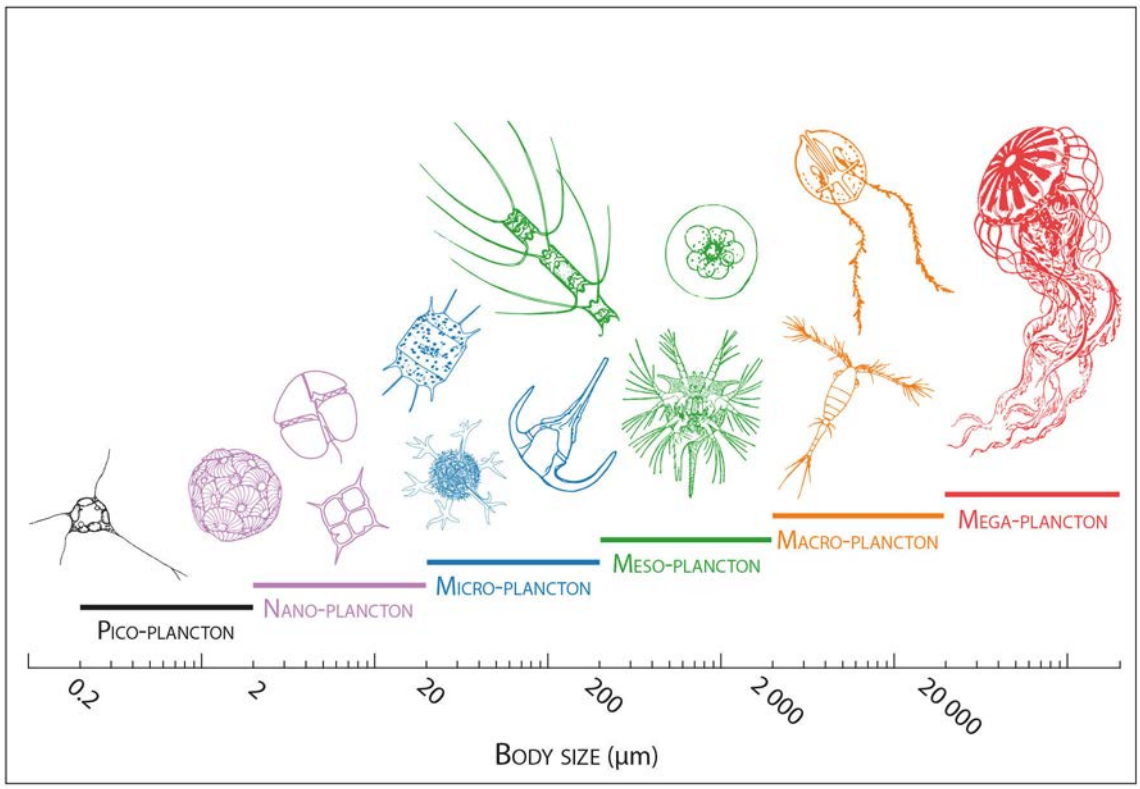

Figure 1 | Schéma conceptuel de la classification du plancton en différentes classes de tailles.

Le phytoplancton, composante photosynthétique de la vie planctonique marine, est exclusivement composé de cellules végétales vivant à la surface des océans, dans la couche euphotique. Même s'ils ne représentent que 1 à 2% de la biomasse végétale de la Terre, ces organismes microscopiques marins sont responsables de près de la moitié de la fixation annuelle et globale de carbone (Field et al., 1998). En plus d'agir directement sur le climat

terrestre en absorbant le dioxyde de carbone (CO_2 ; Hays et al., 2005), la production du phytoplancton est à la base du développement des niveaux trophiques supérieurs. En effet, ces producteurs primaires marins peuvent être par la suite consommés par le zooplancton, qui constitue la composante animale du plancton. La matière organique va ainsi se propager dans la chaîne trophique, depuis le zooplancton herbivore, en passant par les carnivores, pour finir en ressource nutritive pour les grands prédateurs parmi lesquels l'Homme constitue le haut de la chaîne trophique (Figure 2 ; Fenchel, 1988). Une partie de la matière organique produite par le phytoplancton est également transformée en pelotes fécales après son ingestion par le zooplancton herbivore. Ces pelotes fécales vont ensuite sédimenter dans la colonne d'eau, entraînant ainsi un export de carbone en profondeur (Urrère et Knauer, 1981). Cette composante biologique de l'export du carbone, entraînée par l'action du zooplancton, et connue sous le nom de « pompe biologique », représente une fraction importante du cycle globale de carbone (Wilson et al., 2013).

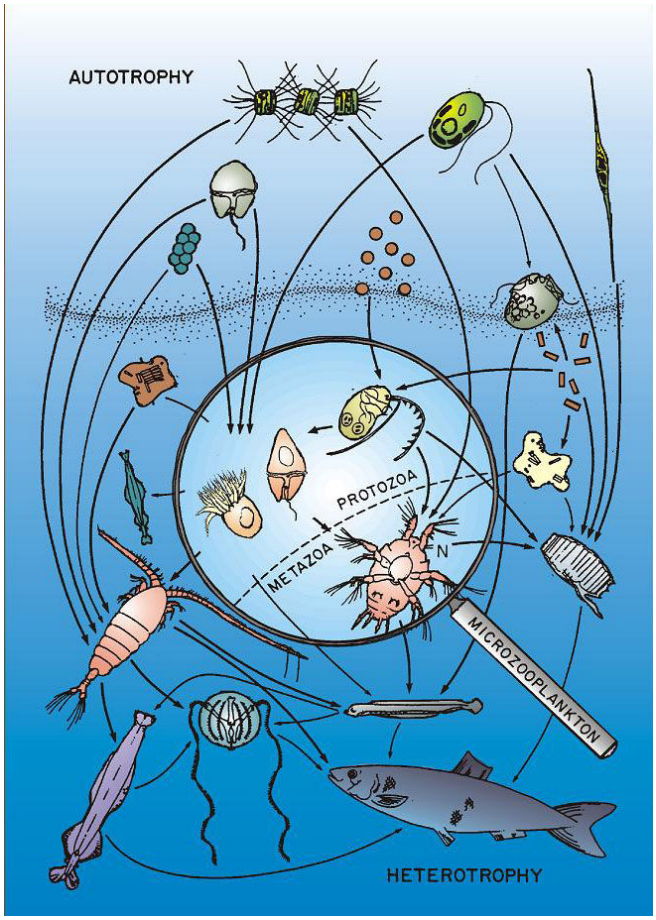

Figure 2 | Chaîne trophique marine d'après Conover (1982).

Le phytoplancton et le zooplancton ont tous deux des rôles prépondérants dans les océans. Ces deux composants de la vie marine fournissent de nombreux services éco-systémiques allant du recyclage de nutriments (carbone, nitrate, etc.), et de la séquestration du carbone atmosphérique au support des pêcheries. Dans un contexte récent de changements climatiques globaux (IPCC, 2014), de pressions anthropiques grandissantes (Halpern et al., 2008) et notamment de leurs effets sur les océans (ex : augmentation de température, acidification), le phytoplancton et le zooplancton sont tous deux des indicateurs potentiels des changements induits par ces pressions (Attrill et al., 2007 ; Beaugrand et Kirby, 2010 ; Hallegraeff, 2010 ; Richardson, 2008). Face à ces pressions grandissantes, il est aujourd'hui indispensable de mieux caractériser la biodiversité marine afin de mieux comprendre l'impact qu'auront ces changements sur les écosystèmes marins.

Depuis les premières grandes expéditions naturalistes visant à explorer les océans, telle que celle du Challenger à la fin du XIX^{ème} siècle et qui fut une des expéditions fondatrices de l'océanographie moderne, les naturalistes n'ont eu de cesse de mieux décrire la biodiversité marine. Cependant, là où les patrons de diversité terrestre sont généralement bien définis (Gaston, 2000), notre compréhension de la structuration et répartition de la biodiversité marine reste encore incomplète (Morin et Fox, 2004). À l'échelle globale des océans, l'étude de la distribution du phytoplancton indique une décroissance de la diversité vers les hautes latitudes (Barton et al., 2010). Ce patron de diversité qui peut être également appliqué aux écosystèmes terrestres (Hillebrand, 2004) ne semble pas être limité au phytoplancton dans les océans, mais concerne également le zooplancton ou les mammifères marins (Figure 3). Bien qu'il ne soit pas aisé de déterminer des facteurs biotiques ou abiotiques pouvant expliquer les

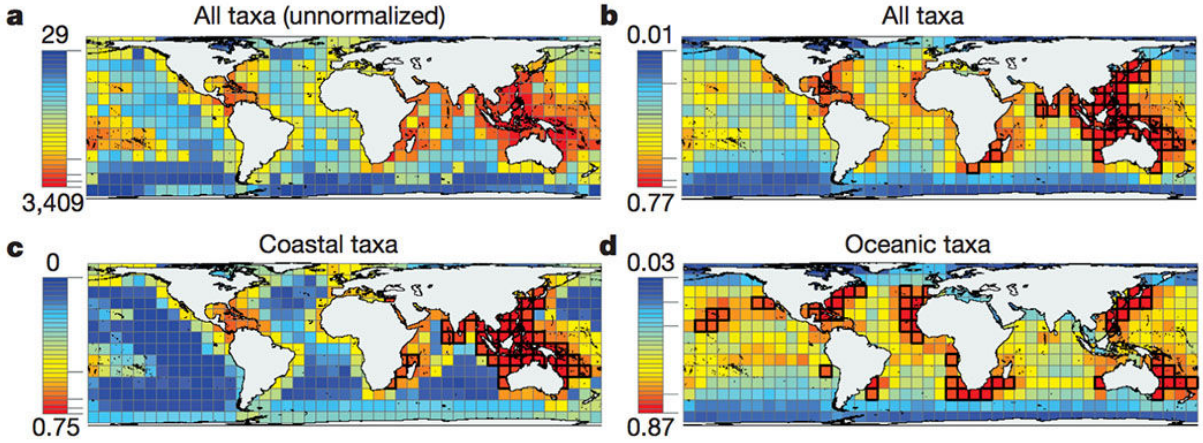

Figure 3 | Distribution globale de la richesse spécifique des océans. (a) Richesse spécifique marine globale, (b) richesse spécifique normalisée, (c) richesse spécifique des taxons côtiers et (d) des taxons océaniques. Les cellules surlignées indiquent les points-chauds de richesse spécifique. Les marques horizontales sur les échelles latérales indiquent les différents quartiles (Tittensor et al., 2010).

patrons de diversité dans les océans, la température semble jouer un rôle important dans la structuration de la biodiversité marine, que ce soit pour le phytoplancton ou le zooplancton (Rombouts et al., 2009 ; Tittensor et al., 2010). De plus, l'étude de la biodiversité planctonique a montré à l'échelle globale une variation le long d'un gradient de biomasse (Irigoien et al., 2004). Ainsi, que ce soit pour le phytoplancton ou le zooplancton, la diversité est une fonction unimodale de la biomasse. Pour de très faibles et très fortes valeurs biomasses, la diversité sera minimale, là où elle sera maximale pour des biomasses moyennes. Cette relation unimodale peut s'illustrer dans le cas des efflorescences massives (ou « bloom ») de micro-algues photosynthétiques telles les diatomées, où l'augmentation soudaine de biomasse se fait au détriment de la diversité (Huisman et al., 1999). Grâce aux données satellitaires et la quantification de la chlorophylle *a*, un *proxy* de la biomasse phytoplanctonique, il est possible de cartographier la distribution en surface du phytoplancton au sein des océans (Figure 4). Cette cartographie nous permet de distinguer les régions à forte biomasse aux hautes latitudes, en régions côtières et autour de l'Equateur, des régions à très faibles biomasses, notamment au centre des grands gyres océaniques que l'on trouve au niveau des latitudes tropicales (Figure 4). Ce patron de distribution est sensiblement identique pour le zooplancton (Strömberg et al., 2009). Cependant, la dépendance de la biodiversité

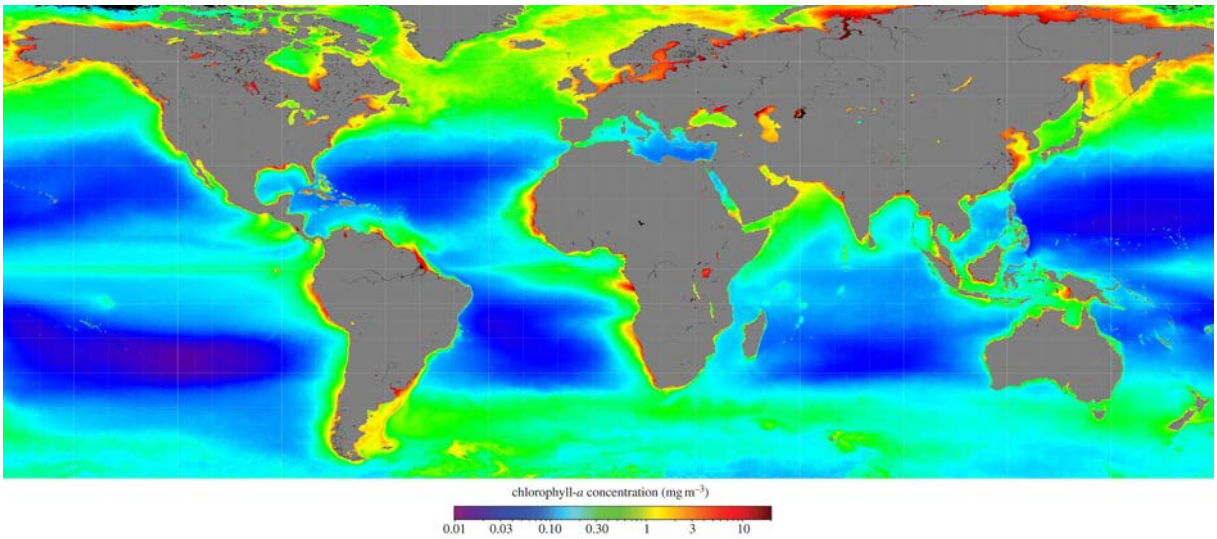

Figure 4 | Distribution de biomasse phytoplanctonique à partir de concentration de chlorophylle *a* satellitaire (© Nasa).

planctonique vis-à-vis de sa biomasse est aujourd'hui remise en question à travers la prise en compte des espèces rares. Facilement sous-estimée avec des méthodes d'échantillonnage standards (filets à plancton ou bouteille Niskin), ces espèces rares pourraient représenter une part significative de la diversité, tout en ne formant qu'une part infime de la biomasse (Vallina et al., 2014). De plus, à cause des biais inhérents aux méthodes d'échantillonnage classiques (faible volume échantillonné, dommages aux organismes, etc.), le rôle et l'importance de nombreux organismes fragiles sont souvent sous-estimés voire ignorés (Remsen et al., 2004). Ainsi, la majorité de nos connaissances sur l'écologie du plancton, en particulier du zooplancton, repose sur un certain nombre d'organismes facilement collectés et dont l'étude n'est pas contrainte par l'impossibilité de les maintenir en culture (comme c'est le cas pour de nombreux taxons planctoniques). Ce phénomène se vérifie notamment pour les copépodes, petits crustacés marins souvent considérés comme les organismes les plus abondants du zooplancton et maillons intermédiaires entre la production primaire et les niveaux trophiques supérieures (Figure 2), ou encore des euphausiacés, organismes emblématiques des écosystèmes antarctiques (Le Fèvre et al., 1998 ; Turner, 2004). Il existe cependant un ensemble d'autres organismes, eucaryotes ou procaryotes, parfois de grandes dimensions (>1 mm) dont l'importance écologique n'a été révélée que récemment.

1.2. Les Rhizaria, un super-groupe de protistes méconnus

Bien que la classification des Eucaryotes fasse constamment l'objet de nouvelles études et d'évolutions, elle permet de distinguer une série de super-groupes tels que les Archaeplastida, les Excavata, les Amoeboza, les Opisthokonta, mais aussi le super-groupe SAR (pour Stramenopila, Alveolata, Rhizaria ; Figure 5). En raison du caractère incultivable de la plupart des taxons qui le composent, les études portant sur la diversité des Rhizaria, ses interactions avec les autres super-groupes eucaryotiques, sont particulièrement complexes. De plus, contrairement à la majorité des autres groupes d'eucaryotes marins, il n'y a que très peu de données, morphologiques ou moléculaires, à disposition (Figure 5), ce qui a amené certains

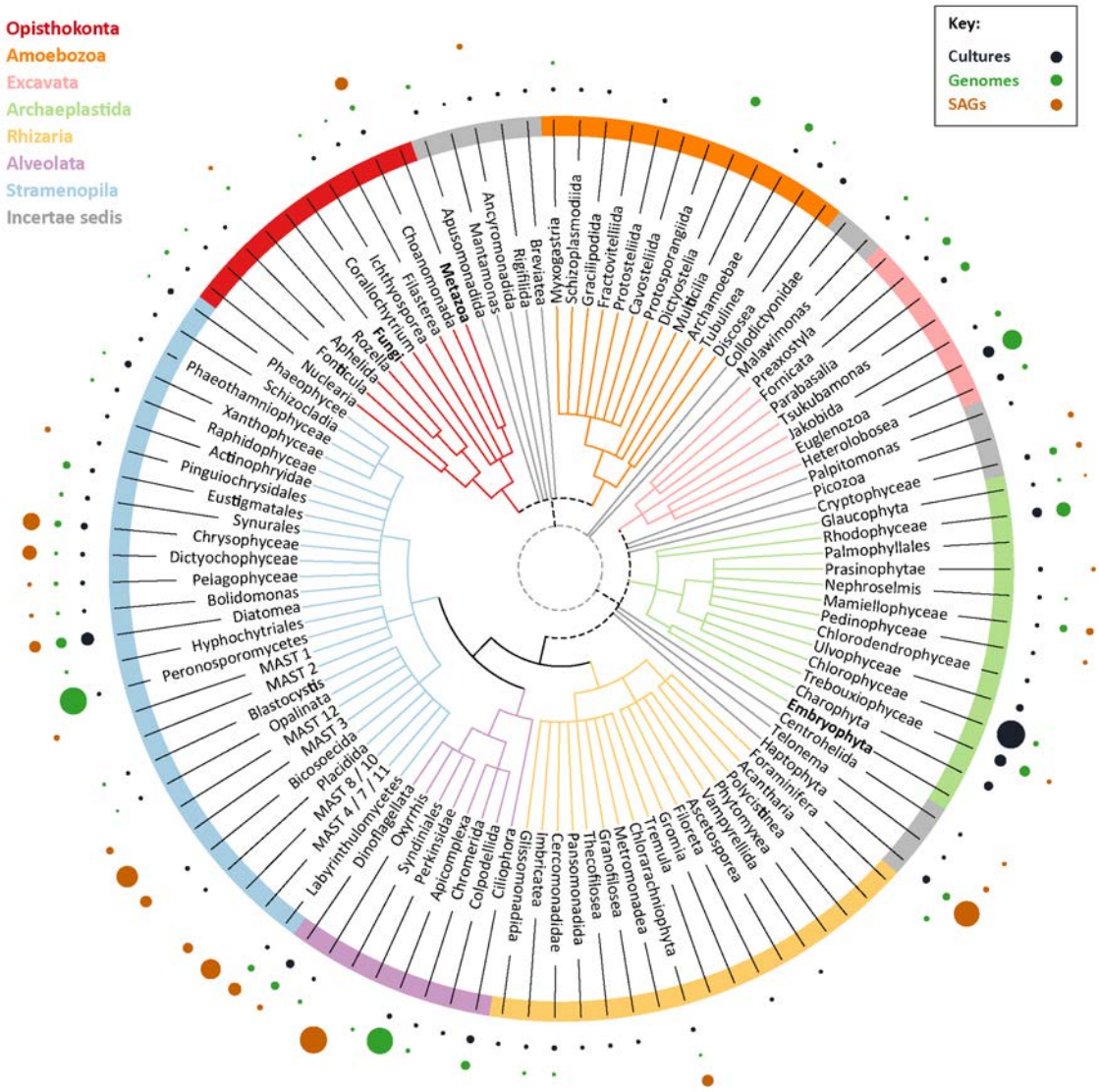

Figure 5 | Arbre schématique des lignées eucaryotes. Pour chaque groupe sont indiqués 1) le nombre de cultures disponibles, 2) le nombre de génomes extraits de la banque de donnée GOLD et 3) le nombre de génomes de cellules uniques (Single Amplified Genomes). La taille des points est proportionnelle au nombre d'espèces. Les Fungi et les Métazoaires sont exclus de l'analyse. Modifié d'après Del Campo et al. (2014).

scientifiques à caractériser les Rhizaria comme l'un des groupes d'eucaryotes parmi les moins étudiés à ce jour (Burki et Keeling, 2014).

Auparavant désignés sous le terme de *« Sarcodines »* ou *« Sarcomastigophores »*, deux termes désormais invalides d'un point de vue taxinomique, mais encore fréquemment utilisés en océanographie, les Rhizaria constituent un groupe diversifié dont la classification a évolué au cours des dernières années. Il existe deux sous-embranchements parmi les Rhizaria, où se distinguent les Cercozoa et les Retaria (Moreira et al., 2007). Bien que la plupart des Rhizaria soient hétérotrophes, on trouvera au sein des Cercozoa, les deux seuls sous-groupes contenant des Rhizaria photosynthétiques : les Chlorarachniophytes et les espèces appartenant au genre *Paulinella* (Lauterborn, 1895 ; McFadden et al., 1994). Les Cercozoa sont des

protistes caractérisés par une forme amiboïde, par la présence de flagelles et dont la grande diversité en milieu marin n'a été révélée que récemment (Bass et Cavalier-Smith, 2004). Au sein des Retaria, les Foraminifères et les Radiolaires sont des protistes hétérotrophes tous deux caractérisés par la présence d'un squelette minéral entourant une cellule amiboïde. La nature de ce squelette (ou test) minéral permet de différencier les Foraminifères, possédant un squelette fait de calcite, des Radiolaires possédant un squelette en silice ou en sulfate de strontium. Apparus respectivement à l'orée de l'ère Primaire (Cambrien inférieur, ~540 m.a.) et au Jurassique (~180 m.a.), les Radiolaires et les Foraminifères planctoniques comptent parmi les lignées de protistes les plus anciennes et les plus diversifiées (Figure 6 ; Suzuki et Oba, 2015). Grâce à leur squelette minéral pouvant sédimenter au fond des océans, Radiolaires et Foraminifères constituent tous deux des groupes particulièrement étudiés par la communauté paléontologiste en tant que marqueurs biostratigraphiques couramment utilisés dans les reconstructions des paléoenvironnements (LaRiviere et al., 2012 ; Wever et al., 2002). Enfin, bien qu'ils soient tous deux fréquemment étudiés en micropaléontologie, seuls les Foraminifères ont bénéficié de recherches approfondies portant sur leur diversité actuelle et leur écologie en milieux marins.

Le terme « Radiolaire » a pendant longtemps été utilisé pour regrouper tout protiste possédant un squelette fait de silice. Pourtant, cette seule caractéristique n'apparaît désormais plus comme étant un solide critère de classification et, après de nombreuses controverses, cinq groupes principaux semblent finalement faire consensus (Figure 7 ; Suzuki et Not, 2015). On distingue au sein des Radiolaires : (1) les Acanthaires, caractérisés par leur squelette fait de sulfate de strontium (Figure 8a) ; (2) les Taxopodides, eux, caractérisés par une multitude de spicules silicifiés, formant des panaches d'axopodes (Figure 8b) ; et enfin (3) les Polycystines, regroupant Collodaires, Nassellaires et Spumellaires, tous trois caractérisés par un squelette de silice (Figures 8c-e ; Suzuki et Aita, 2011). Bien qu'originellement intégrés au sein des Radiolaires, les Phaeodaires ont été récemment transférés parmi les Cercozoa, grâce à l'apport des reconstructions phylogénétiques (Nikolaev et al., 2004 ; Yuasa et al., 2005). C'est également aux bénéfices des outils moléculaires, que l'on doit la présence des Taxopodides au sein des Radiolaires, longtemps considérés comme appartenant aux Héliozoaires (Sierra et al., 2013). Dans les phylogénies récentes, les différents groupes de Radiolaires forment systématiquement des groupes monophylétiques, quoique leurs relations soient encore largement hypothétiques (Decelle et al., 2012 ; Krabberød et al., 2011 ; Kunitomo et al., 2006).

Avec plus de 700 espèces, les Radiolaires sont des protistes hétérotrophes distribués de façon ubiquitaire dans les océans, de la surface aux régions abyssales (Suzuki et Not, 2015). Ils couvrent un vaste spectre de taille allant du nano- au macro-zooplancton, soit presque 6 ordres de grandeur. L'ensemble des espèces de Radiolaires est exclusivement composé de protistes marins bien qu'il existe une espèce, le Nassellaire *Lophophaena rioplatensis*, qui a pu être observée dans les eaux saumâtres d'un estuaire sud-américain (Boltovskoy et al., 2003). Parmi les Radiolaires vivants dans les zones photiques, plusieurs études décrivent la présence de microalgues symbiotiques (photosymbiontes) vivant au sein de certaines espèces (Anderson, 2012). La diversité des partenaires symbiotiques inclut des

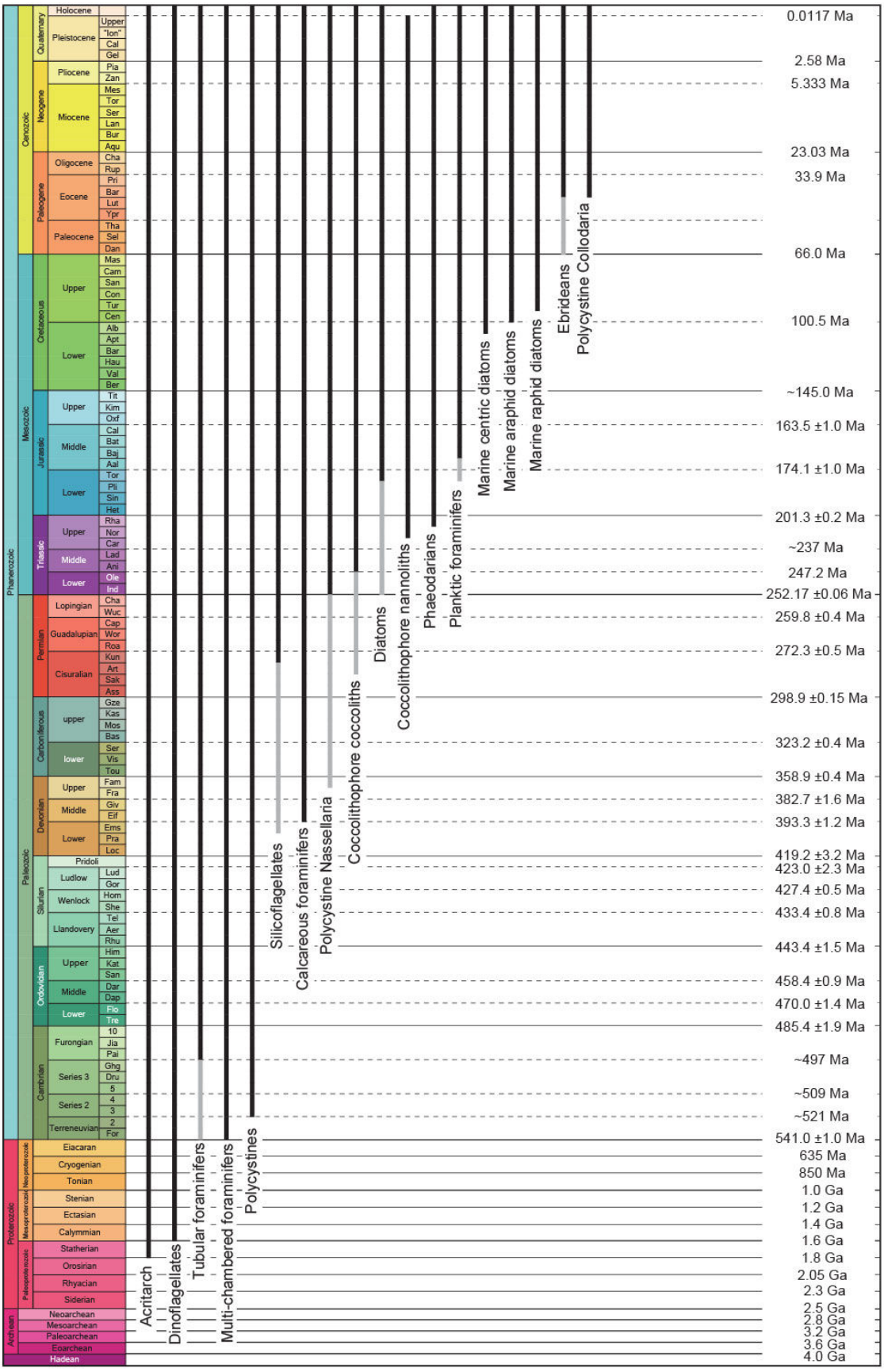

Figure 6 | Histoire géologique des protistes marins fossiles. *Lignes noires* : étendue des données fossiles pour chaque taxon. *Lignes grises* : étendue incertaines des données fossiles. Modifié d'après Suzuki et Oba (2015).

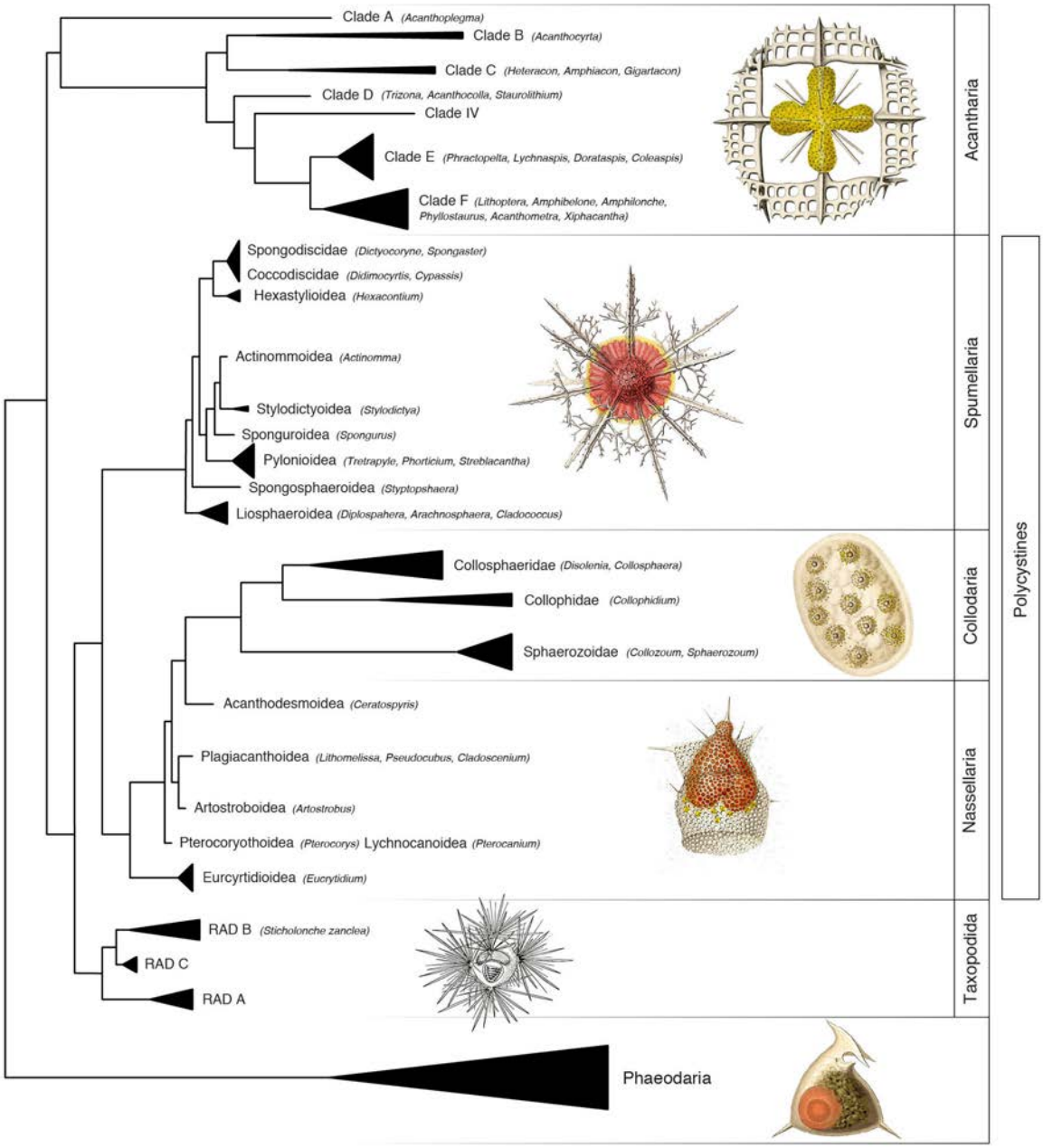

Figure 7 | Arbre schématique des Radiolaires. Les relations entre chaque ordre sont basées sur les connaissances morpho-moléculaires actuelles. La taille des branches correspond au nombre d'espèces vivantes. Les Phaeodaires, appartenant aux Cercozoaires, sont placés comme « outgroup ». Modifié d'après Suzuki et Not (2015).

cyanobactéries (Yuasa et al., 2012), des dinoflagellés (Anderson et al., 1998) mais aussi des Prymnésiophytes et des Prasinophytes (Anderson et al., 1983 ; Decelle et al., 2012). La diversité et l'abondance des Radiolaires dans les écosystèmes marins varient grandement en fonction du groupe concerné. Les Acanthaires forment un groupe très diversifié et figurent parmi les Radiolaires les plus abondants en milieu océanique (Decelle et al., 2012, 2013 ; Michaels, 1988 ; Michaels et al., 1995). Les Sticholonches, bien que localement très abondants (~10 000 cellules m^{-3}), ne sont aujourd'hui composés que d'une seule espèce, *Sticholonche zanclea*, mais pourraient néanmoins abriter une diversité beaucoup plus large (Not et al., 2007 ; Tan et al., 1978). Parmi les Polycystines, seuls les Nassellaires et les

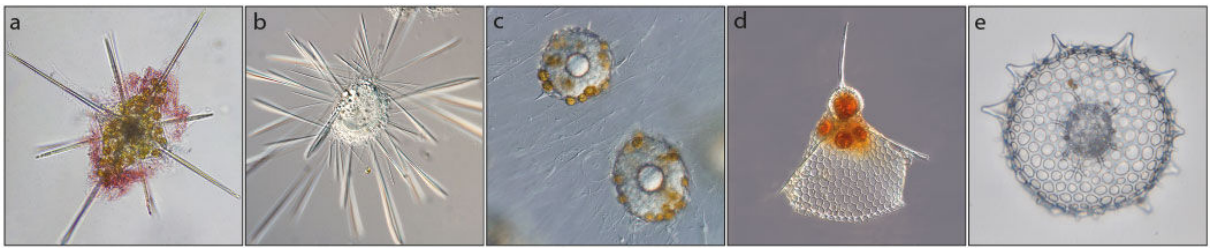

Figure 8 | Illustrations des différents ordres de Radiolaires. (a) Un Acanthaire, (b) un Taxopodide, (c) Détail de deux capsules centrales d'une colonie de Collodaire, (d) un Nassellaire (© J. Dolan) et (e) un Spumellaire (© N. Suzuki)

Spumellaires font l'objet d'études détaillées, portant notamment sur leur signature biostratigraphique (Boltovskoy et al., 2010), sur l'ontogénie et la morphogénèse de leur squelette (Anderson et al., 1989 ; Matsuoka, 1992) ou encore sur des mécanismes de prédation (Matsuoka, 2007 ; Sugiyama et al., 2008). Enfin, parmi les trois ordres de Polycystines et plus globalement au sein de tous les Radiolaires, les Collodaires apparaissent comme étant les moins étudiés à ce jour, que ce soit vis-à-vis de leur classification, leur biologie, leur écologie ou encore de leur importance dans les océans modernes.

2. Les Collodaires, état des connaissances

2.1. Morphologie, classification et évolution

Morphologie

Les Collodaires ont développé une morphologie complexe et unique vis-à-vis des autres Radiolaires. Ils sont ainsi le seul groupe parmi les Radiolaires ayant des individus coloniaux (Suzuki et Not, 2015). L'analyse détaillée de la morphologie d'une cellule de Collodaire au sein d'une colonie révèle une compartimentation des organelles qui se partagent entre l'endoplasme et le cytoplasme. Le terme de « capsule centrale » peut être préféré pour parler de chaque cellule unique, bien que ce terme ne désigne techniquement que la partie endoplasmique (ou région intra-capsulaire) qui contient le(s) noyau(x), les mitochondries, des structures cristallines et parfois des gouttelettes lipidiques (GL ; Figure 9). La partie ectoplasmique (ou région extra-capsulaire) est, elle, constituée de fins et longs rhizopodes formant un réseau dense où chaque cellule semble être connectée aux cellules adjacentes (Rh, Figure 9). Au sein de ce réseau cytoplasmique, se côtoient algues symbiotiques, proies, vacuoles digestives et autres structures granulaires (Figure 9) (Anderson, 1976a). Des vacuoles (ou alvéoles), parfois de grandes tailles, sont incluses dans la matrice du Collodaire (Vc, Figure 9) et, même si leur composition chimique est inconnue, elles pourraient permettre à la colonie de réguler sa flottaison (Anderson, 1976b ; Gamble, 1909).

La majorité des colonies présente une forme cylindrique, ellipsoïdale ou sphérique, mais des formes plus complexes sont fréquemment observées (ex : enchevêtrement des

Figure 9 | Morphologie d'une colonie de Collodaire : (Cc) capsule centrale, (GL) gouttelette lipidique, (Rh) rhizopode, (Sy) photosymbionte et (Vc) vacuole ou alvéole.

différentes colonies longilignes ; Figure 10a). Chaque colonie est composée de dizaines, voire milliers, de capsules centrales qui s'agglomèrent dans une matrice gélatineuse (Figure 10a). La taille des colonies varie de quelques millimètres à plusieurs centimètres (avec un maximum de trois mètres reporté dans la littérature ; Swanberg et Harbison, 1979). Les capsules centrales sont quant à elles de bien plus petite taille (20 – 300 µm) et leur nombre moyen au sein d'une colonie a été estimé à ~9 capsules centrales par mm^2 (ce chiffre étant variable en fonction de l'état physiologique de la colonie, ou en fonction de son stade de développement ; Dennett et al., 2002). Tantôt sphérique ou ovoïde, la forme des capsules centrales diffère au sein d'un même individu ou selon les espèces (Figure 9). Chez certaines espèces, les capsules centrales sont caractérisées par une multitude de fins spicules, également faits de silice et très similaires à ceux des éponges, qui parsèment leur périphérie (Figure 10b). D'autres espèces présentent des capsules centrales entourées d'un squelette de silice dont la taille et la forme sont variables d'une espèce à l'autre (Figure 10c). Enfin, contrairement à l'ensemble des Radiolaires qui possèdent tous un squelette, certains Collodaires ne présentent pas de structure siliceuse quelle qu'elle soit autour de leurs capsules centrales (Figure 9 - image de droite). Ces Collodaires dénudés de toutes structures siliceuses sont plus couramment décrits comme étant des Collodaires nus.

Alors que la majorité des espèces de Collodaires sont coloniales, certaines sont unicellulaires (Figure 10d). Ces Collodaires dits « solitaires » possèdent une morphologie générale différente des colonies. La plupart étant de grande taille (millimétriques), ces individus solitaires sont organisés autour d'une seule capsule centrale de grande taille (>300 µm). À la différence des capsules centrales d'un individu colonial qui peuvent contenir plusieurs noyaux, celle des Collodaires solitaires est mononuclée (Suzuki et al., 2009). Cependant, malgré ces quelques différences notoires, l'analyse plus fine de leur anatomie souligne de nombreuses similarités avec les individus coloniaux (Anderson, 1976c). Ainsi, tout comme certaines espèces de Collodaires coloniaux, la présence de spicules et plus rarement de squelette, permet de caractériser quelques espèces de Collodaires solitaires (Figure 10e). De nombreuses alvéoles, similaires à celles trouvées dans les colonies, entourent la capsule centrale et occupent, chez certaines espèces, jusqu'à deux tiers du volume de l'organisme. Il est également fréquent de voir s'étendre le réseau de rhizopodes, bien au-delà

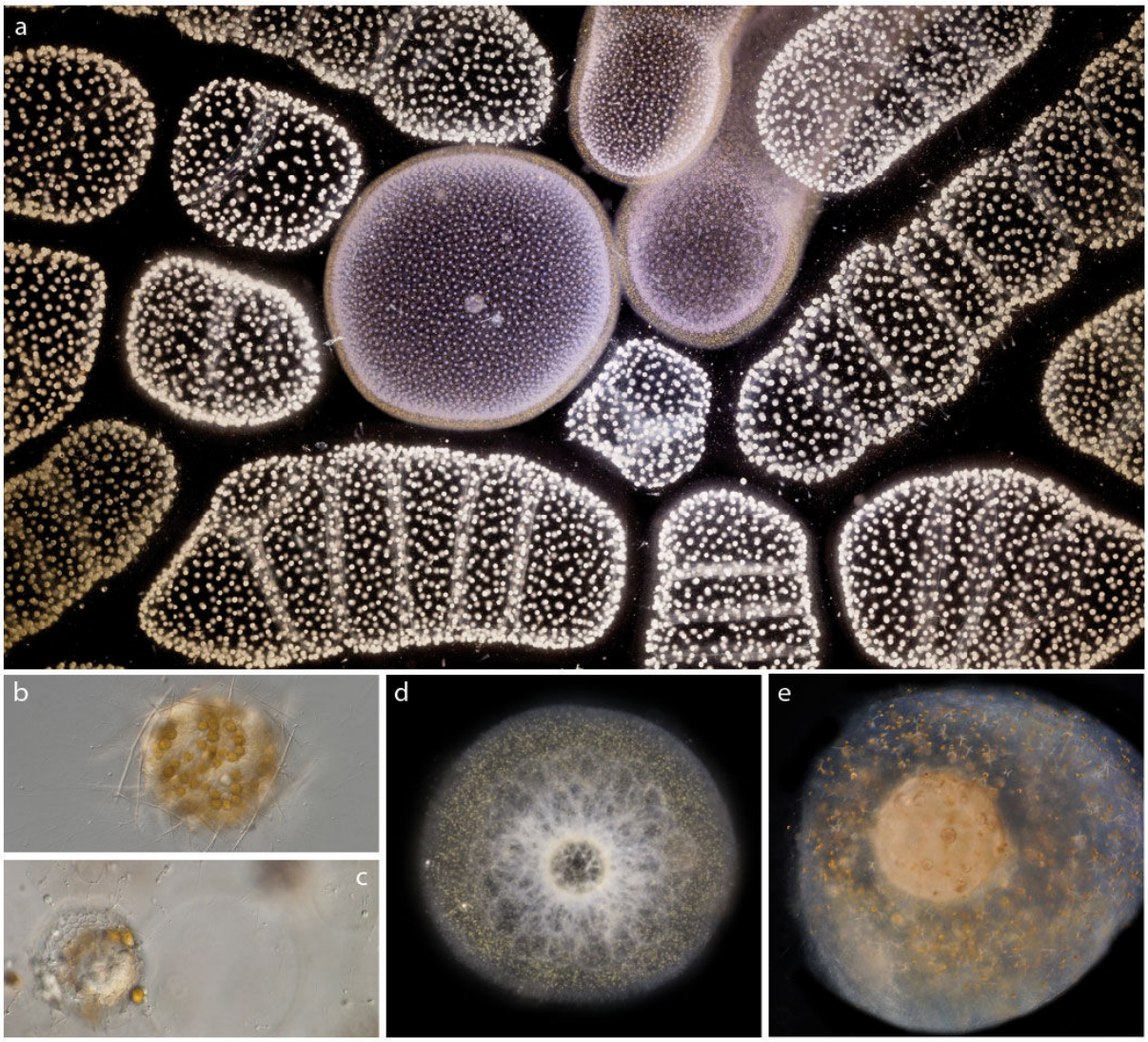

Figure 10 | Illustrations des différentes morphologies de Collodaires. (a) Plasticité morphologique des colonies de Collodaires (© C. Sardet). (b) Détail d'une capsule centrale de colonie entourée de spicules. (c) Détail d'une capsule centrale de colonie entourée d'un squelette. (d) Un Collodaire solitaire. (e) Un Collodaire solitaire possédant quelques spicules.

de la périphérie de ces alvéoles (Figures 10d-e). Enfin, ces individus solitaires possèdent également un nombre important d'algues symbiotiques distribuées dans ce réseau de rhizopodes particulièrement dense (Figures 10d-e).

Classification

Les Collodaires ont été historiquement séparés en trois familles, principalement en prenant en compte la présence ou l'absence de formes coloniales (Figure 11). Les Thalassicollidae (Haeckel) forment une famille exclusivement composée d'individus solitaires où chaque espèce est différenciée en fonction de sa forme générale, de la position de ses alvéoles ou de la présence de spicules dans la matrice extra-capsulaire (Anderson et al., 2002). Plusieurs genres ont été définis au sein des Thalassicollidae, mais le manque de critères taxonomiques fiables ne permet pas d'estimer correctement le nombre de genres (et accessoirement d'espèces). Les Collosphaeridae (Müller) regroupent tout individu colonial

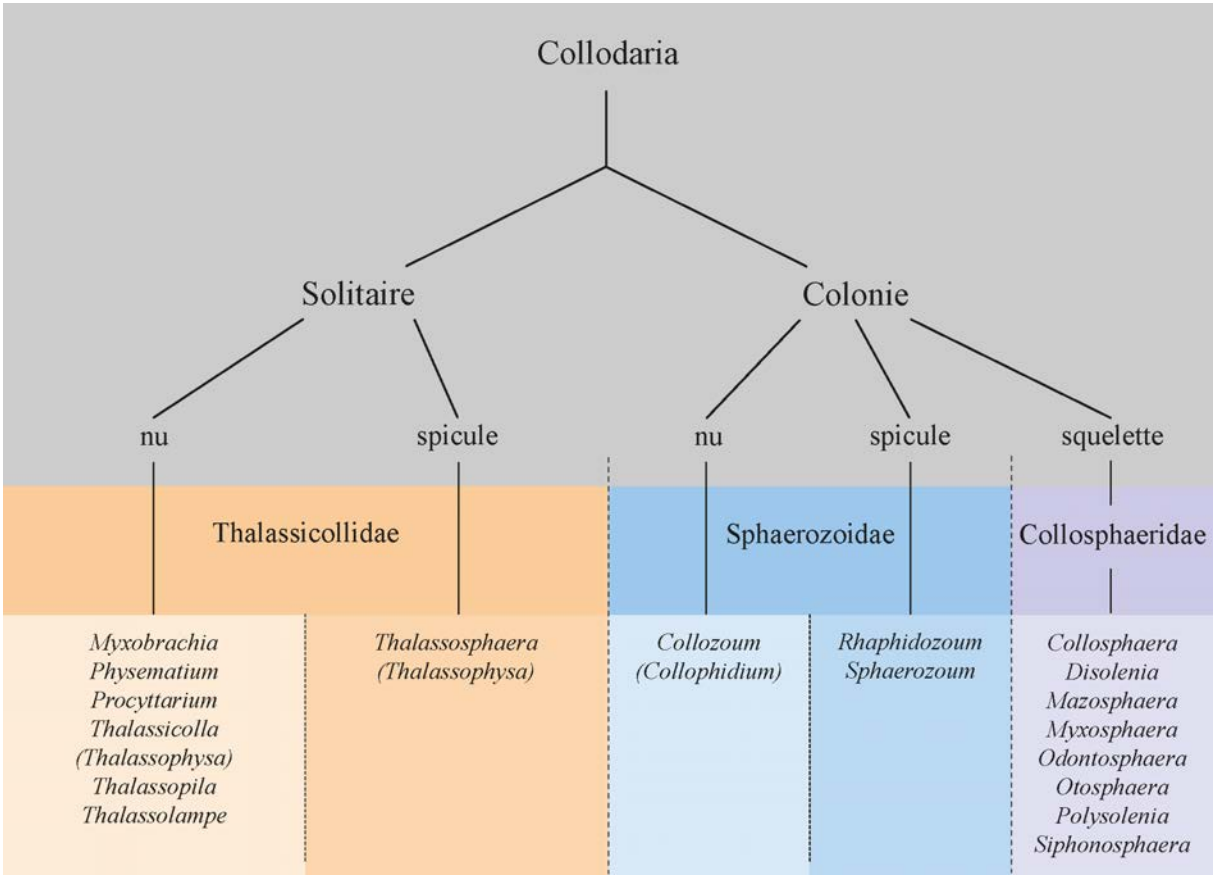

Figure 11 | Illustration de la clé d'identification taxonomique des Collodaires d'après Haeckel (1887), Anderson et al. (2002), Suzuki et Aita (2011). Les noms de genre entre parenthèses soulignent leur placement ambiguë.

possédant des capsules centrales entourées d'un squelette. Chaque espèce appartenant aux Collosphaeridae est définie grâce à la morphologie du squelette. Bien que la majorité des Collodaires passent inaperçus dans les études de paléostratigraphie, les Collosphaeridae sont les seuls Collodaires à être préservés sous forme fossile dans les sédiments marins (Suzuki et Oba, 2015). On daterait l'apparition des premiers Collodaires fossiles à l'orée du Cénozoïque (43-65 m.a. ; Haslett, 2004 ; Wever et al., 2002). Cette préservation a permis des études détaillées de la morphologie du squelette des Collosphaeridae, mais aussi de définir plusieurs critères taxinomiques (ex : taille et forme du squelette, nombre et positions de pores, présence d'extensions périphériques) permettant une identification taxinomique plus précise (Figure 12a-b' ; Strelkov et Reshetnyak, 1971). Il existerait une cinquantaine d'espèces de Collosphaeridae, se répartissant dans 11 genres différents (pers. comm. Dr. Noritoshi Suzuki). Enfin, les Sphaerozoidae (Müller) forment une famille de Collodaires exclusivement coloniaux dont les capsules centrales sont nues ou entourées de spicules. À cause de leur grande fragilité et de leur ressemblance avec les spicules d'éponges, l'analyse des spicules fossiles dans les sédiments est particulièrement difficile. Tout comme les squelettes de Collosphaeridae, l'étude de la morphologie et de la position des spicules permet de différencier les espèces de Sphaerozoidae à spicules. Il existe deux formes principales de spicules : 1) des spicules tri-radiés (Figure 12c-c') et 2) des spicules simples en forme d'aiguilles (Figure 12d-d'). Ces deux formes sont caractéristiques des deux seuls genres de

Sphaerozoidae à spicules : les *Sphaerozoum* et *Rhaphidozoum*, respectivement. Les Collodaires nus appartenant aux Sphaerozoidae sont quant à eux beaucoup plus difficiles à identifier sur la base de la morphologie. En effet, le manque de critères taxinomiques accessible en microscopie optique ne permet pas de séparer aisément les différentes espèces au sein des Collodaires nus (Brandt, 1885 ; Haeckel, 1887). L'analyse de l'ultrastructure de ces Collodaires nus a néanmoins permis de définir deux genres grâce à la densité des vacuoles cytoplasmique et notamment grâce à la forme de la capsule, sphérique ou ovale pour les espèces appartenant au genre *Collozoum* (Figure 9), ou de forme serpentines pour le genre *Collophidium*.

Face aux contraintes imposées par une approche taxinomique classique, l'utilisation des méthodes moléculaires et l'analyse des variations du code génétique ont permis de fournir de nouveaux outils permettant de s'affranchir de la morphologie et d'identifier efficacement les espèces. Au cours des vingt dernières années, l'utilisation de la phylogénie moléculaire appliquée à différents groupes de protistes marins, a permis de corriger un certain nombre de schémas de classification établis à travers les analyses morphologiques. Chez certains groupes de Rhizaria (ex : Acanthaires, Phaeodaires, etc.) ces approches moléculaires ont mis en évidence de nombreuses différences avec les classifications traditionnelles et ont permis de définir de nouveaux critères de classification plus robustes (Decelle et al., 2012, 2013 ; Nakamura et al., 2015 ; Scoble et Cavalier-Smith, 2014). Ces méthodes ont ainsi entraîné l'établissement de nouveaux cadres de références morpho-moléculaire, où chaque espèce est définie par une série de critères morphologiques mais également en prenant en compte sa signature moléculaire. La signature moléculaire étant unique pour chaque espèce génétique, elle peut être utilisée comme un outil d'identification taxonomique. De ce constat naquit le concept de *barcode* (littéralement code-barres), motivé par la nécessité d'identifier un

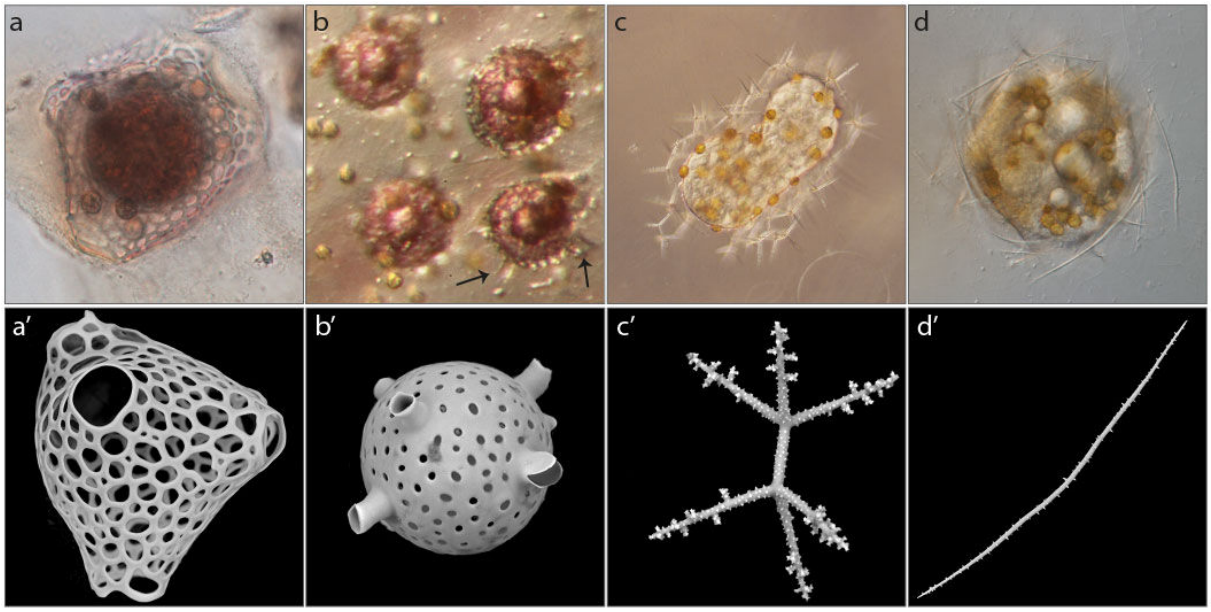

Figure 12 | Illustration des différentes formes de structures en silice observable chez les Collodaires. (a,a') *Disolenia zanguebarica* et son squelette aux larges pores. (b-b') *Siphonosphaera abyssi* et son squelette aux longues extensions. (c-c') *Sphaerozoum armatum* et ses spicules tri-radiés aux multiples extensions micrométriques. (d-d') *Rhaphidozoum acuferum* et ses spicules simples.

spécimen en comparant sa signature moléculaire avec celles des espèces connues (Hebert et al., 2003). Il faudra attendre la fin du XX^{ème} siècle pour voir publiées les premières séquences moléculaires attribuées aux Collodaires (Zettler et al., 1997). Dans cette première phylogénie, dédiée à l'étude générale des Radiolaires, le séquençage du gène ribosomal 18S a mis en évidence le positionnement basal d'une séquence de *Thalassicolla nucleata* (espèce appartenant à la famille des Collodaires solitaires Thalassicollidae) vis-à-vis de trois autres espèces coloniales, suggérant ainsi une origine évolutive des Collodaires axée sur les individus solitaires. Dans les années qui suivent la publication de ce premier article, de nouvelles séquences sont venues enrichir la base de données moléculaires naissante et ont contribué à l'élaboration de phylogénies moléculaires plus robustes (Zettler et al., 1999, 1998). Ces premières phylogénies confirment ainsi la monophylie des trois familles de Collodaires, déjà reconnue dans les schémas de classification taxinomique, même si les auteurs souligneront néanmoins la difficulté de tirer des conclusions au vu du nombre limité de séquences. Par la suite, quelques études vont contribuer ponctuellement à améliorer la phylogénie des Collodaires (Ishitani et al., 2012 ; Krabberød et al., 2011 ; Kunitomo et al., 2006 ; Polet et al., 2004 ; Yuasa et al., 2005). Ces études ont notamment confirmé l'existence des Collodaires, comme étant un ordre à part entière et non inclus dans les Spumellaires, comme cela a longtemps été suggéré (Wever et al., 2002). Plus récemment, l'existence d'une quatrième famille, les Colliphidae, a été suggérée via l'analyse du gène ribosomal 18S (Ishitani et al., 2012). Néanmoins, le manque flagrant de séquences de références (22 séquences pour le gène ribosomal 18S et 2 pour le gène 28S) ne permet pas d'établir des relations claires entre les différentes familles, genres ou espèces de Collodaires. En outre, il est impératif de pouvoir relier chacune de ces séquences moléculaires à des critères morphologiques afin de pouvoir comparer les deux méthodes de classifications.

Évolution

L'étude de la morphologie des Collodaires a permis peu à peu de proposer des hypothèses concernant l'évolution de cet ordre au cours du temps. Ces hypothèses ont été principalement émises suite à l'étude des rares Collodaires fossiles, appartenant aux Collosphaeridae (Boltovskoy et al., 2010). C'est notamment l'analyse de la structure des éléments siliceux chez les Collodaires (ex : squelette ou spicules) qui a conduit certains auteurs à affirmer que le squelette serait le résultat d'une fusion de plusieurs spicules (Anderson et Swanberg, 1981 ; Strelkov et Reshetnyak, 1971). Cette hypothèse a néanmoins été réfutée à cause du manque de données fossiles permettant d'étayer la relation entre les Collosphaeridae et leurs ancêtres supposés, les Sphaerozoidae (Bjorklund et Goll, 1979). Plus récemment, l'étude détaillée des capsules centrales de plusieurs espèces de Collodaires a mis en évidence une différence notoire du nombre de noyaux entre ces différentes familles. Les Thalassicollidae solitaires ne posséderaient qu'un seul noyau là où les deux autres familles de Collodaires pourraient posséder plusieurs noyaux par capsules centrales. Cette différence a conduit les auteurs à proposer un nouveau schéma d'évolution pour les Collodaires, en suggérant qu'ils auraient évolué d'une forme solitaire vers une forme coloniale (Suzuki et al., 2009). Avec le développement des approches moléculaires, les hypothèses concernant l'évolution des Collodaires ont pu être testées sous un tout nouvel angle. Le manque de

relations phylogénétiques robustes a ainsi apporté de nouveaux éléments pour réfuter l'hypothèse concernant l'apparition des squelettes chez les Collodaires (Zettler et al., 1999). Malgré l'apport des outils moléculaires, les schémas d'évolution restent encore incomplets. Une meilleure compréhension de la classification de ces organismes, ainsi que davantage de connaissances sur leur biologie et leur cycle de vie, seraient des éléments d'autant plus importants pour élaborer davantage d'hypothèses concernant l'histoire évolutive des Collodaires.

2.2. Biologie

Cycle de vie

Nos connaissances sur le cycle de vie des Collodaires restent encore très partielles et reposent pour la plupart sur d'anciennes études. De par la nature incultivable de ces protistes marins, il est à l'heure actuelle impossible de suivre l'évolution d'une génération à la suivante. Cette difficulté est notamment liée au manque de connaissances sur la biologie de ces organismes, que ce soit vis-à-vis de leur nutrition ou de leurs préférences écologiques. Néanmoins, en les maintenant en vie sur des périodes de quelques jours (maximum = 34 jours), des études ont permis d'acquérir des informations précieuses pour la compréhension du cycle de vie des Collodaires (Swanberg et Anderson, 1985). C'est à la toute fin du XIX^{ème} siècle que Karl Brandt, zoologiste allemand de renom et pionnier de la recherche sur les Collodaires, fit les premières observations des mécanismes reproductifs chez des individus coloniaux (Brandt, 1885). Il observa au sein de plusieurs espèces coloniales (ex : *Collozoum inerme*, *Sphaerozoum neapolitanum*), des divisions cellulaires au sein des capsules centrales. Ces divisions, appelées aussi couramment « fissions binaires », sont décrites comme étant un événement de reproduction asexuée, où une cellule mère se divise, donnant naissance à deux cellules filles. De tels mécanismes reproductifs sont couramment observés chez d'autres grands protistes marins à test minéral tels que les Tintinides (Dolan, 2013) où les Foraminifères (Goldstein, 2003). En réussissant à maintenir quelques Collodaires en vie dans son laboratoire, Brant observa que de telles divisions avaient également lieu chez trois espèces de Collodaires solitaires, en particulier *Thalassophysa sanguinolenta* (Brandt, 1902). L'étude détaillée du noyau de cette dernière espèce lui fit suggérer que cette forme solitaire pouvait donner naissance à une nouvelle colonie (ou « proto-colonie ») par une simple succession de divisions binaires (Figure 13b). Cette proto-colonie, identifiée comme l'espèce coloniale *Collozoum pelagicum*, établit le premier lien entre une espèce solitaire et une espèce coloniale, suggérant que ces deux formes pourraient faire partie d'un même cycle de vie. Cette identité commune fut confirmée beaucoup plus tardivement par l'apport des méthodes moléculaires qui montreront une parfaite identité moléculaire entre les deux formes (Polet et al., 2004 ; Zettler et al., 1999). Enfin, près de 50 ans après les observations de Karl Brant, l'étude de cette même espèce solitaire a montré que, en plus de pouvoir donner naissance à une colonie, elle pouvait à un moment donné de son développement, former une multitude de petites cellules flagellées (~10 µm) appelées « spores » ou « swarmers » (Figure 13b', e) (Hollande et Enjumet, 1953). Cette production de cellules biflagellées, certes observée auparavant (Brandt, 1885 ; Huth, 1913), n'est pas sans rappeler celle observée chez les

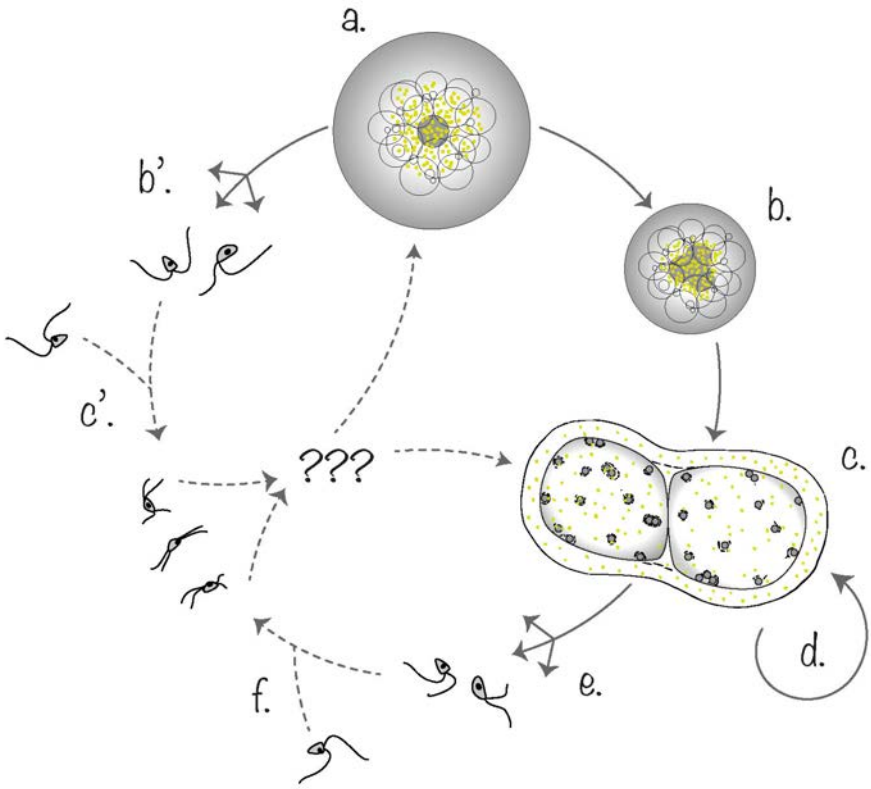

Figure 13 | Illustration schématique du cycle de vie hypothétique des Collodaires d'après Hollande et Enjumet (1953). Les traits pointillés illustrent les liens hyphotétiques. **Cycle asexué :** un Collodaire solitaire (a) peut se diviser par fission binaire (b) pour former une colonie (c) qui elle même continue de se développer et grandir par fission binaire (d). **Cycle sexué :** un Collodaire solitaire (a) ou colonial (c), peut entrer en mitose pour produire une multitude de cellules flagellées, *swarmers* (b', e). Ces *swarmers* pourraient fusionner entre eux (c', f), mais leur devenir est inconnu.

Phaeodaires (Borgert, 1909) ou les Acanthaires (Decelle et al., 2013 ; Febvre, 1977). Bien que leur ploïdie n'ait jamais pu être étudiée, ces spores pourraient vraisemblablement être des gamètes sexuels suggérant l'existence d'une reproduction sexuée chez les Collodaires. Au vu des différents éléments présents dans une littérature majoritairement ancienne, le cycle de vie des Collodaires semble être partagé entre reproduction asexuée et sexuée. Cependant, eu égard à l'impossibilité de les cultiver, il est impossible de confirmer les hypothèses précédemment émises. Comprendre le cycle de vie des Collodaires permettrait de mieux interpréter les données environnementales qui suggèrent, entre autres, la présence de Collodaires dans les profondeurs.

La Photosymbiose

La photosymbiose, association mutualiste entre un organisme photosynthétique et son hôte, est une interaction biotique répandue dans les écosystèmes marins (Stoecker et al., 2009). De telles interactions, associant de nombreuses espèces de Radiolaires à divers partenaires photosynthétiques (photosymbiontes), ont été couramment décrites (Anderson, 1983, 2012). Parmi l'ensemble de ces Radiolaires, la totalité des espèces de Collodaires

connue à ce jour est exclusivement photosymbiotique (Swanberg, 1979). Le nombre de partenaires symbiotiques au sein des Collodaires varie avec la taille de l'organisme hôte. On compte une centaine, voire plusieurs milliers, de symbiontes au sein d'une seule colonie ou d'un seul individu solitaire (Figure 14 ; Caron et Swanberg, 1990). Depuis la description de *Zooxanthella nutricola*, première espèce de symbionte décrite chez un Collodaire (Brandt, 1882a), une seule autre espèce symbiotique (un Prasinophyte, *Pedinomonas symbiotica*) a été décrite chez un Collodaire solitaire (Anderson, 1976 ; Cachon et Caram, 1979). L'utilisation quasi systématique du terme générique *zooxanthelle* dans la littérature dédiée aux Collodaires, limite néanmoins nos capacités à interpréter la véritable diversité de ces partenaires. Ce n'est que récemment que l'apport des outils moléculaires a permis l'étude plus détaillée des symbiontes (Gast et Caron, 1996). Le séquençage du gène ribosomal 18S des symbiontes au sein de cinq espèces distinctes de Collodaires a ainsi mis en évidence la présence d'une même et unique espèce de symbionte (génétiquement similaire au dinoflagellé *Scrippsiella nutricula*). Néanmoins, le nombre limité de spécimens de Collodaires étudiés permet difficilement d'extrapoler la présence de cette seule espèce de dinoflagellé à l'ensemble des espèces de Collodaires.

Dès les premières observations *in vivo* de la photosymbiose à la fin du XIX^{ème} siècle, les scientifiques ont essayé de comprendre la nature de cette association et l'importance qu'elle pouvait avoir au sein de l'hôte (Brandt, 1882b ; Cienkowski, 1871). Au cours d'une simple expérience opposant des Collodaires solitaires photosymbiotiques, les uns incubés à l'obscurité, les autres incubés à la lumière du soleil, Karl Brant (1882) observa la capacité des Collodaires incubés à la lumière de pouvoir survivre sur une période de temps bien plus longue que les Collodaires à l'obscurité. Il fut ainsi le premier à souligner l'importance des symbiontes dans la survie des Collodaires et suggéra alors leur rôle nutritif. Près d'un siècle plus tard, l'utilisation du radiomarqueur ^{14}Carbone a permis d'étudier les échanges de composés photosynthétiques au sein du complexe hôte-symbionte et de mettre en évidence une translocation de composés organiques dérivés de la photosynthèse, des symbiontes vers

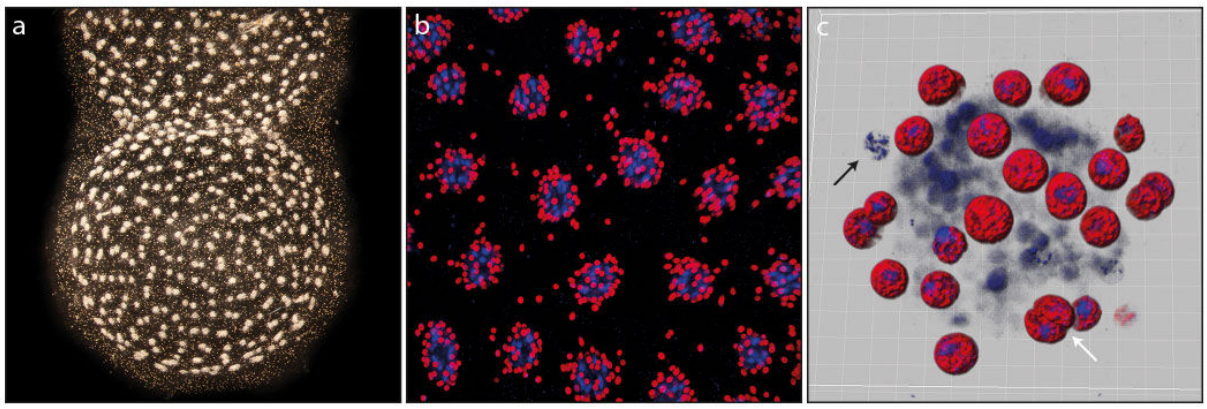

Figure 14 | Illustrations de la photosymbiose chez les Collodaires coloniaux. (a) Une colonie et ses milliers de photosymbiontes (les petites cellules dorées). (b) Image en microscopie confocale montrant l'organisation des photosymbiontes (en rouge) au sein d'une colonie et leur étroite association avec les différentes cellules de Collodaires (en bleu) (© S. Colin). (c) Détail d'une cellule de Collodaire colonial (bleu) et de ses photosymbiontes associés (rouge). La flèche blanche indique une cellule de photosymbionte en division. La flèche noire indique une cellule sénescente de photosymbionte, dont seul reste le noyau (© S. Colin).

les capsules centrales de l'hôte (Anderson, 1978a). Malgré les bénéfices sous-jacents de la translocation des produits dérivés de la photosynthèse, il semblerait que l'hôte puisse également se nourrir périodiquement de ses propres symbiontes (Anderson, 1976b). Bien que les paramètres qui influencent l'ingestion des symbiontes restent encore inconnus, le nombre constant de symbiontes observé dans un Collodaire au cours du temps suggère que le complexe hôte-symbiontes reste stable. L'ingestion pourrait ainsi permettre d'éliminer les cellules sénescentes (Figure 14c) tout en libérant de l'espace pour de nouveaux symbiontes. Néanmoins, la diminution du nombre de symbiontes lors de la phase précédant la formation des swarmers conduit à deux hypothèses : 1) l'ingestion continue des symbiontes pour fournir les ressources nécessaires au processus reproductif ; ou 2) le relargage de la totalité des symbiontes. Bien qu'il y ait peu d'arguments en faveur de l'un ou l'autre des deux mécanismes, la question de la spécificité se pose. En effet, alors que les symbiontes soient capables de se diviser au sein même de la colonie (Figure 14c), le relargage des symbiontes suggère qu'il n'y a pas de transmission verticale entre la cellule mère et la cellule fille. Dans le cas des Collodaires, le cycle de vie suggère qu'il y aurait une transmission horizontale des symbiontes, à savoir une acquisition des symbiontes en phase libre (Anderson, 2012). Cette transmission horizontale impliquerait donc des mécanismes de reconnaissance spécifiques, encore inconnus pour le moment.

La présence de microalgues photosynthétiques au sein des Collodaires permet à ces derniers de contribuer à la production primaire des écosystèmes marins. Les expériences de radiomarquage ont ainsi permis d'estimer la productivité des Collodaires *via* leurs partenaires symbiotiques (Caron et al., 1995 ; Swanberg, 1979 ; Swanberg et Harbison, 1979). En dépit du fait que de nombreux paramètres abiotiques (ex : température, illumination, etc.) et/ou biotiques (ex : état physiologique, stade développement, etc.) soient susceptibles de faire varier la productivité des symbiontes, celle-ci semble être constante entre les individus solitaires et coloniaux, et elle représente en moyenne une production de 360 ngC par organisme et par heure (Anderson, 1983 ; Caron et al., 1995). Grâce à ces estimations de production primaire mesurées *in vivo*, quelques études ont pu reporter localement la contribution des Collodaires à la productivité des écosystèmes dans lesquels ils se trouvent. Bien que leur contribution puisse être importante localement, par exemple dans le Golfe d'Aden où Khmeleva (1967) estima que les Collodaires pouvaient produire davantage de matière organique que la totalité du phytoplancton analysé dans le même environnement, celle-ci reste néanmoins le plus souvent anecdotique (~1%) à l'échelle de la production primaire d'un écosystème (Caron et al., 1995 ; Dennett et al., 2002 ; Khmeleva, 1967 ; Swanberg, 1979). Malgré cette mince contribution, la production primaire des Collodaires photosymbiotiques est unique de par la fraction de taille (meso- et macro-zooplancton) dans laquelle elle a lieu et une estimation plus globale permettrait de mieux appréhender son impact réel sur les écosystèmes marins.

Nutrition et prédation

La place des Collodaires au sein des chaînes trophiques marines est encore incertaine. En plus d'abriter des partenaires photosymbiotiques, qui sont susceptibles d'être parfois

utilisés comme source nutritive (Anderson, 1976b), les Collodaires peuvent également consommer des proies capturées à la périphérie de leur matrice. En raison des difficultés à maintenir ces organismes en culture, les quelques expériences réalisées sur la nutrition des Collodaires n'ont pu couvrir qu'une courte période de temps (Anderson, 1978b ; Swanberg, 1979 ; Swanberg et al., 1986 ; Swanberg et Anderson, 1985). À travers ces expériences, une multitude de proies ont été néanmoins observées : tintinides (Figure 15a), copépodes (Figure 15b), larves de mollusques, diatomées, mais aussi d'autres radiolaires, suggérant que les Collodaires sont globalement des omnivores. Une fois les proies capturées dans le réseau de pseudopodes, elles vont être engluées progressivement au sein de la matrice gélatineuse où elles seront fractionnées en micro-particules et finalement transportées vers l'endoplasme (Anderson, 1978b). Cependant, les auteurs de ces études mentionnent qu'il est difficile de déterminer s'il s'agit d'une prédation active ou passive. En effet, de par la nature particulièrement collante de la matrice gélatineuse entourant les Collodaires, il est difficile de déterminer si les organismes capturés ne sont pas juste englués dans ce piège gélatineux et digérés par la suite (Anderson, 1983). De plus, les Collodaires n'étant pas capables de se mouvoir activement dans l'eau, ils apparaissent plus comme des prédateurs opportunistes se nourrissant d'une large gamme de proies, rencontrées de manière aléatoire.

Peu de prédateurs des Collodaires ont été décrits à ce jour. Lors de ses expériences *in vivo* sur des Collodaires, Neil Swanberg essaya de nourrir des larves de poissons avec quelques colonies (Swanberg, 1979). Toutes les larves tentèrent bien de manger les colonies mais, à sa grande surprise, ces larves ne s'en nourrirent pas après un premier contact avec les colonies. Il fut proposé par la suite que les photosymbiontes présents dans ces colonies étaient la cause de cette répulsion (Anderson, 1983). En effet, grâce à la production de substances tel que le stérol, connu pour être un synthétisé par les dinoflagellés (Withers, 1983), les Collodaires pourraient dégager une « odeur » nauséabonde pour les prédateurs. Néanmoins, d'autres preuves indiquent que les Collodaires peuvent être consommés par divers crustacés tels des amphipodes hypérides (Figure 15c ; Swanberg, 1979), des copépodes, ou même des larves de homard (O'Rorke et al., 2012). Il y a donc peu d'éléments prouvant que les Collodaires sont des proies préférentielles dans les communautés planctoniques. Ce désintérêt

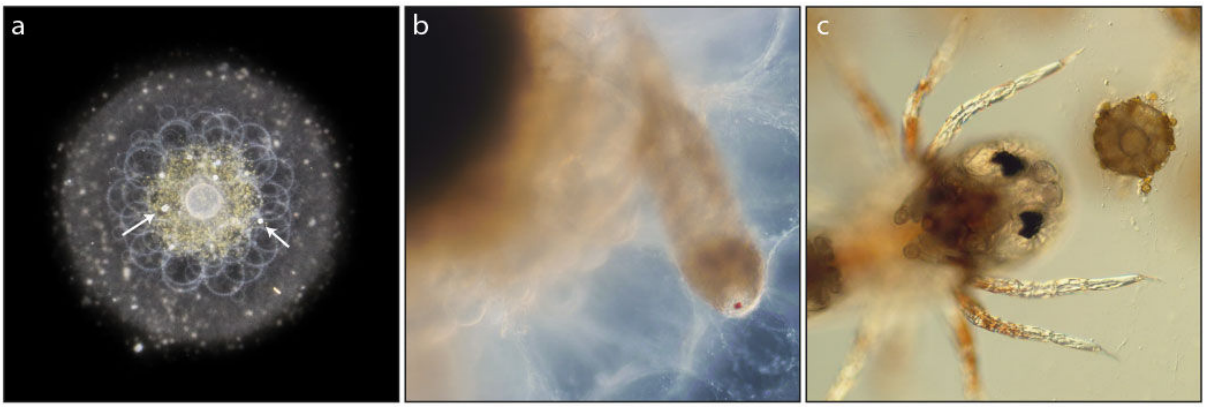

Figure 15 | Illustrations des proies et des prédateurs des Collodaires. (a) Un Collodaire solitaire ayant ingéré de nombreux tintinides (indiqués par les flèches blanches). (b) Détail de la matrice extracellulaire d'un Collodaire solitaire où est englué un copépode calanoïde. (c) Un amphipode hypéride observé au sein même de la matrice d'une colonie.

des Collodaires pourrait refléter différents mécanismes permettant à ces organismes de se protéger des prédateurs, que ce soit grâce à leurs symbiontes, leur grande taille ou à la nature collante de leur matrice extracellulaire.

2.3. Biogéographie et abondances

Il n'existe que peu de données permettant de cartographier la distribution des différentes espèces de Collodaires. Il faut attendre la fin des années 1960 pour voir publiées quelques études rapportant sporadiquement la diversité des Collodaires dans plusieurs régions océaniques (Pavshtiks et Pan'kova, 1966 ; Strelkov et Reshetnyak, 1971). Dans chacune de ces études, l'utilisation systématique de méthodes classiques d'échantillonnage (ex : filets à plancton) a grandement limité notre compréhension de la distribution biogéographique des espèces de Collodaires. En effet, les biais techniques inhérents à l'utilisation de tels outils d'échantillonnage, les perturbations mécaniques causant des dommages irréversibles aux organismes fragiles ou le faible volume d'eau échantillonné, n'ont permis d'acquérir que des données parcellaires dans l'étude des Collodaires. Néanmoins, l'étude de 42 espèces collectées des tropiques aux eaux froides des hautes latitudes a mis en évidence que seules quelques espèces semblaient être endémiques, là où la grande majorité se sont révélées être ubiquistes (Strelkov et Reshetnyak, 1971). Cette étude a notamment permis de montrer qu'une majorité d'espèces pouvait être trouvée ensemble dans les eaux chaudes des tropiques. À l'opposé, cette même étude a mis au jour que la diversité des Collodaires décline notoirement dans les eaux tempérées et froides. Ce patron de distribution a également été rapporté *via* l'analyse des sédiments marins (Boltovskoy et al., 2010). En effet, l'utilisation des carottes de sédiments marins pour l'étude de la diversité des Collodaires a également rendu possible une cartographie de la répartition de quelques espèces à partir des fossiles laissés au fond des océans (Figure 16). Néanmoins, le succès de cette technique repose uniquement sur la conservation d'une trace fossile ; elle n'a donc pas autorisé l'étude des Collodaires dépourvus de structures siliceuses. Ainsi, seuls les Collodaires possédant un squelette (c'est-à-dire appartenant à la famille des Collosphaeridae) ont pu être étudiés, mais ni les Collodaires nus, ni les Collodaires à spicules n'ont pu être observés grâce aux données sédimentologiques. De plus la Figure 16 illustre le manque flagrant de données acquises pour la colonne d'eau *via* des filets à plancton et des pièges à sédiments. Ainsi, pour les Collodaires possédant une trace fossile, la grande majorité des données de diversité à disposition provient des sédiments marins et non d'échantillons de plancton. Malgré les biais d'échantillonnage dus aux filets à plancton et mentionnés précédemment, de précieuses données ont été apportées par la suite grâce aux travaux novateurs de Neil Swanberg. En effet, la collecte *in situ* de Collodaires, prélevés par des plongeurs à l'aide de jarres en verre, a permis d'améliorer considérablement l'étude de ces organismes fragiles (Swanberg, 1979). Grâce à cette approche, plusieurs nouvelles espèces de Collodaires ont pu être décrites (Swanberg et Anderson, 1981 ; Swanberg et Harbison, 1979). Cependant, cette technique étant particulièrement coûteuse en temps, son application a été limitée à une dizaine d'expéditions océanographiques majoritairement réparties dans l'Atlantique Nord (Swanberg, 1979).

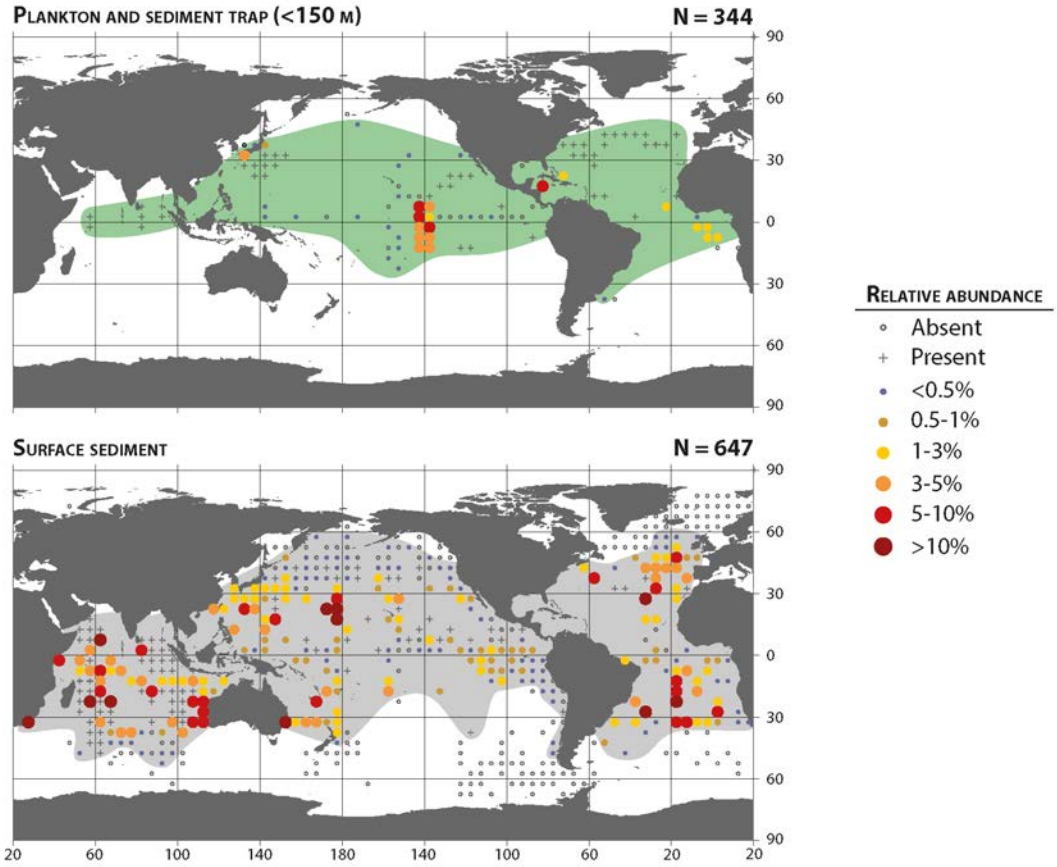

Figure 16 | Biogéographie du Collodaire *Acrosphaera spinosa* à partir de 1) les données acquises dans la colonne d'eau grâce aux filets à plancton et aux pièges à sédiments (N = 344) et 2) les sédiments de surface (N = 647). Modifié d'après Boltovskoy et al. (2010).

Les études de diversité réalisées sur des échantillons de plancton à partir de l'identification morphologique des spécimens requièrent une expertise taxinomique souvent longue et difficile à acquérir. Dans le cas des Collodaires, viennent s'ajouter le manque de critères taxinomiques fiables et la difficulté à différencier les espèces entre elles, limitant ainsi considérablement les analyses de diversité. Face à ce constat que l'on retrouve dans bon nombre de groupes planctoniques différents, l'utilisation des outils moléculaires a permis de révolutionner l'approche de la biodiversité planctonique. L'analyse de la diversité environnementale, *via* la création de librairies de clones, notamment celles portant sur l'analyse du gène 18S de l'ARN ribosomal, fut une des premières approches permettant de diminuer considérablement le temps nécessaire à l'identification des espèces dans un échantillon (Díez et al., 2001 ; López-García et al., 2001 ; Moon-van der Staay et al., 2001). En plus d'un gain de temps dans l'analyse des échantillons, cette approche a révélé une diversité encore inconnue dans de nombreux groupes de plancton marin, et notamment chez les Radiolaires (Countway et al., 2007 ; Edgcomb et al., 2002, 2011 ; Not et al., 2007 ; Sauvadet et al., 2010 ; Yuan et al., 2004). La plupart de ces études ont généré des séquences pour la fraction de taille du pico-plancton (0,8 - 5 µm), fraction dans laquelle il est peu probable de trouver des Collodaires, qui, eux, appartiennent plus couramment au méso- et macro-zooplancton. Plusieurs hypothèses ont été émises depuis pour tenter d'expliquer la présence de ces séquences : (1) l'existence d'une diversité beaucoup plus large et

potentiellement ignorée dans la petite fraction de taille ; (2) la présence de cellules flagellées (swarmers) portant la signature moléculaire des Collodaires dans les petites fractions de taille ; (3) la destruction de plus grandes cellules, fractionnées, dont l'ADN se retrouve dans les petits fractions de taille ; ou (4) l'existence d'ADN extracellulaire (Not et al., 2007, 2009). De plus, ces séquences affiliées aux Collodaires ont souvent été observées en grand nombre dans les librairies de clones (Edgcomb et al., 2011 ; Massana, 2011 ; Not et al., 2009 ; Sauvadet et al., 2010). Ces résultats soulèvent néanmoins bon nombre de questions au regard de l'état actuel de nos connaissances sur la diversité et l'écologie des Collodaires. Par la suite, le développement de méthodes de séquençage à haut-débit (pyroséquençage 454 puis Illumina) a permis d'accroître la profondeur de séquençage et d'améliorer nos capacités à explorer la diversité planctonique (Edgcomb et al., 2011 ; Lindeque et al., 2013 ; Logares et al., 2014 ; Sogin et al., 2006). L'application de ces méthodes à des échantillons issus de pièges à sédiments, a notamment révélé la contribution potentielle des Collodaires dans les flux de particules (Amacher et al., 2009 ; Fontanez et al., 2015). Enfin, la publication récente de plusieurs études portant sur l'analyse des données de séquençage à haut-débit des échantillons des Expéditions *Tara* Océans (Pesant et al., 2015) et Malaspina (Duarte, 2015), a souligné l'importance des Collodaires dans les écosystèmes marins, qu'ils soient en surface (de Vargas et al., 2015) ou dans les profondeurs des océans (Pernice et al., 2015). Néanmoins, bien qu'elles constituent des outils sans précédents et offrent de nouvelles perspectives dans l'étude des Collodaires, ces méthodes moléculaires restent pour la plupart purement qualitatives et ne permettent qu'une description semi-quantitative de la biodiversité.

Abondances et biomasses

Tout comme l'identification taxinomique, la quantification des abondances et biomasses de Collodaires restent des tâches complexes, tant il est difficile de collecter ces organismes avec des méthodes d'échantillonnage classiques. Au cours des années 1960-70, trois études successives apporteront de premiers éléments pour comprendre la distribution des abondances de Collodaires (Khmeleva, 1967 ; Pavshtiks et Pan'kova, 1966 ; Strelkov et Reshetnyak, 1971). Les deux premières font mention d'abondances de Collodaires coloniaux particulièrement élevées, entre 3 000 – 4 000 et 16 000 – 20 000 colonies m^{-3}, respectivement dans le détroit de Davis (à la limite de la Mer du Labrador) et le Golfe d'Aden. Par la suite, Neil Swanberg nota que de telles densités élevées n'avaient jamais été observées par ailleurs (Swanberg, 1979). Là où ses propres estimations suggéraient des abondances inférieures d'un facteur dix par rapport aux abondances des équipes russes, il observa néanmoins que de grandes quantités de Collodaires coloniaux pouvaient s'accumuler en surface des océans lors de périodes de calme prolongé (Figure 17). Ces agglomérations par temps calme en surface reflèteraient des mécanismes physiques entraînant une augmentation de la densité des Collodaires. C'est notamment le cas des cellules de Langmuir, structures induites par le vent de surface et pouvant entraîner une rétention d'organismes localement en surface, tels que les Collodaires (Caron et al., 1995). La collecte d'organismes coloniaux dans de telles accumulations, appelées aussi *zoöcurrents* par Ernst Haeckel (1887), pourrait donc être responsable des valeurs de densités particulièrement élevées et soulignent également une difficulté supplémentaire dans la quantification des Collodaires. Grâce à ses observations *in*

Figure 17 | Accumulation en surface de Collodaires coloniaux observés en octobre 2014 à l'entrée de la Baie de Villefranche-sur-Mer (© R. Failletaz).

situ lors de plongées sous-marines, Neil Swanberg a élaboré une technique efficace de quantification *in situ* consistant à compter manuellement le nombre d'organismes passant à travers un cercle de 0,5 m^2 (Swanberg, 1974, 1979). Cette technique lui permis d'avoir des quantifications précises des abondances de Collodaires en surface. Malgré son aspect laborieux et des difficultés apparentes à pouvoir la mettre rapidement en place lors d'expéditions océanographiques, cette technique l'autorisa à étudier la distribution des abondances de Collodaires dans plus de 450 stations réparties dans l'ensemble de l'Atlantique Nord (Swanberg, 1979). Il observa des Collodaires dans 89% des cas et remarqua que les abondances les plus élevées se trouvaient en été dans le gyre Nord Atlantique là où les stations côtières présentaient des densités plus faibles tout au long de l'année. Grâce à ses observations *in situ* il put également mettre en évidence les biais d'échantillonnage dus aux accumulations en surface. En effet, pour certaines stations où il put constater de très fortes densités à quelques centimètres de la surface (~540 colonies m^{-3}), il estima que l'abondance de colonies mesurée dans la colonne d'eau n'était que de 0,4 – 1,1 colonies m^{-3}, soit une abondance insignifiante au vu de la situation en surface. Par la suite, grâce à la collecte de Collodaires aux Bermudes et à l'estimation de leur contenu en carbone, Michaels et al. (1995) vont fournir de précieux facteurs de conversion Carbone-Taille pour les Collodaires solitaires et coloniaux. Grâce à ces facteurs de conversion, ils ont estimé la biomasse des Collodaires présents entre la surface et 150 m de profondeur, à 2,8 mgC m^{-2} en moyenne soit une valeur

proche de celle des Acanthaires pour la même masse d'eau. Cependant, ils insisteront sur les biais de leurs estimations liés à leur échantillonnage effectué avec une pompe à eau et des bouteilles Niskin.

Tout comme les Collodaires, la collecte d'organismes planctoniques fragiles constitue un réel défi et malgré une sophistication de plus en plus avancée, l'utilisation de filets à plancton pour la collecte de ces organismes représente un obstacle à leur étude (Remsen et al., 2004). Bien que chaque filet soit construit pour échantillonner le plancton de façon optimale, leur utilisation entraîne dans la plupart des cas des perturbations mécaniques importantes lors de la collecte. Ces perturbations peuvent provoquer des dommages mineurs aux organismes voire entraîner leur destruction. Certains filets peuvent néanmoins être déployés et traînés à de très faibles vitesses (<0,1 m s^{-1}), mais cela au détriment du volume échantillonné et de la couverture spatiale. Il n'existe qu'une poignée d'études permettant de mesurer l'effet réel d'un échantillonnage traditionnel, utilisant des filets à plancton, sur des organismes planctoniques fragiles (Ashjian et al., 2001 ; Benfield et al., 1996 ; Dennett et al., 2002 ; Gallager et al., 1996 ; Norrbin et al., 1996). Dans l'étude la plus complète à ce jour, l'utilisation simultanée d'un filet à plancton et du système d'imagerie SIPPER (Shadowed Image Particle Profiling and Evaluation Recorder - Samson et al., 2001) a permis de comparer les abondances de divers groupes zooplanctoniques en fonction de l'outil d'échantillonnage (Remsen et al., 2004). Afin de pouvoir effectuer une comparaison directe entre les deux outils, le système SIPPER et les filets furent placés en série de manière à échantillonner exactement le même volume d'eau, celui ci passant d'abord dans le faisceau du SIPPER puis directement dans les filets. Le déploiement de cette plateforme d'échantillonnage multiple sur les 100 premiers mètres des eaux du Golfe du Mexique, révéla une sous-estimation colossale des abondances d'organismes fragiles entre les filets et le système SIPPER. Ce dernier autorisa, entre autres, une quantification des abondances de protistes et de Cnidaires 522% et 1200% supérieures aux abondances estimées par les filets. La biomasse totale du meso-zooplancton fût quant à elle estimée à 2 fois inférieure à la biomasse capturée par le système SIPPER. En plus de soulever de nombreuses questions vis-à-vis de l'efficacité des méthodes traditionnelles d'échantillonnage, cette étude révèle la grande utilité des systèmes d'imageries *in situ* dans l'étude du zooplancton.

Le développement récent des techniques d'imagerie *in situ* a donc permis d'accroître considérablement nos capacités à échantillonner une multitude d'organismes usuellement détruits lors de collectes avec des méthodes classiques de prélèvements telle l'utilisation de filets à plancton (Wiebe et Benfield, 2003). Ces approches, permettant d'observer de façon non-intrusive (sans perturbations mécaniques) les organismes directement dans leur habitat, ont notamment abouti à confirmer la présence de micro-agrégations d'organismes planctoniques en surface, mais aussi à souligner l'importance des bactéries diazotrophes du genre *Trichodesmium* dans cette même partie des océans (Davis et al., 1992 ; Davis et McGillicuddy, 2006 ; Guidi et al., 2012 ; Sandel et al., 2015). Au-delà de leur capacité unique à échantillonner une fraction du plancton couramment sous-estimée, les systèmes d'imagerie *in situ* autorisent le plus souvent une grande couverture spatiale et également une meilleure intégration de l'ensemble de la colonne d'eau en enregistrant chaque organisme à une profondeur donnée (là où seuls des filets fermants, tel le MOCNESS, permettent d'accéder à

une résolution verticale fine). De plus, l'innovation technologique dans les méthodes d'imagerie *in situ* s'est accompagnée d'un développement de l'automatisation des méthodes d'identification taxinomique (Benfield et al., 2007 ; Culverhouse et al., 2006). Ces méthodes de tri semi-automatique ont permis de traiter la grande quantité d'images fournies par les systèmes d'imagerie, mais ont aussi plus globalement abouti à réduire le temps nécessaire à l'identification taxinomique d'organismes au sein d'un échantillon, sans pour autant réduire la qualité de l'identification (Culverhouse et al., 2003). Néanmoins, comme tout outil d'échantillonnage, quel qu'il soit, la gamme d'étude des systèmes d'imagerie *in situ* est également limitée par une série de contraintes technologiques inhérentes à la conception même de l'instrument. Ainsi, d'un système d'imagerie à l'autre, le volume imagé (c'est-à-dire le volume échantillonné) sera différent et la résolution du pixel sera plus ou moins fine, contraignant ainsi la gamme de taille observable. L'exemple peut-être le plus marquant est celui de l'ichtyoplancton (fraction du plancton contenant notamment les larves et juvéniles de poissons), présent dans la grande fraction de taille (meso- et macro-plancton) et qui ne dépasse rarement des densités supérieures à 0,01 individus par litre. Là où les premiers systèmes d'imagerie *in situ* n'avaient qu'un faible volume échantillonné (~1 – 10 L s^{-1}), l'élaboration de l' *IS*IIS (*In Situ* Ichtyoplankton Imaging System) augmente considérablement le volume imagé (>70 L s^{-1}) et ouvre ainsi de nouvelles perspectives dans l'étude *in situ* de ce compartiment planctonique (Cowen et Guigand, 2008). En plus de d'autoriser l'étude de l'ichtyoplancton, l'*IS*IIS a également rendu possible l'observation du plancton gélatineux (ex : Cnidaires, Siphonophores, etc.), un compartiment du macro-zooplancton jusqu'alors extrêmement complexe à étudier avec des filets à plancton tant ceux-ci endommageaient considérablement ces organismes fragiles (Luo et al., 2014).

De nos jours, il existe très peu d'études d'imagerie *in situ* sur les Rhizaria et les Radiolaires saisis dans leur milieu naturel. Une seule étude s'est spécifiquement intéressée au Collodaires (Dennett et al., 2002). Grâce à l'utilisation du VPR (Video Plankton Recorder), un des premiers systèmes d'imagerie *in situ*, cette étude a révélé la présence de fortes abondances de Collodaires coloniaux en surface, dans la partie nord de l'Océan Pacifique. Ces estimations acquises *via* une quantification non invasive des Collodaires étaient supérieures à n'importe quelle estimation précédemment réalisée dans la même région (Kling et Boltovskoy, 1995). De plus, grâce à la détermination du nombre de capsules centrales, utilisé ici comme un *proxy* du nombre de cellules dans chaque colonie et l'application d'un facteur de conversion Carbone-Surface spécifique au Collodaires coloniaux (Michaels et al., 1995), ils ont pu quantifier la biomasse de ces organismes. Leurs résultats suggèrent que les Collodaires coloniaux pourraient contribuer jusqu'à 10% de la biomasse microbienne (pour une fraction de taille supérieure à 2 µm). Au-delà de l'importance qu'elle a pu révéler localement dans l'écosystème Nord Pacifique, cette étude a permis plus largement de mettre en avant l'efficacité des systèmes d'imagerie *in situ* dans la quantification effective des Collodaires. À la différence de ces travaux réalisés localement, l'utilisation au cours de 12 campagnes océanographiques de l'UVP (Underwater Video Profiler), un système d'imagerie *in situ* rendant possible la quantification d'organismes plus grands que 600 µm (Picheral et al., 2010), a offert une vision beaucoup plus globale des patrons de distributions du plancton et des Rhizaria en particulier (Stemmann et al., 2008). À cette échelle de taille et pour une

masse d'eau intégrée entre 100 et 1 000 m de profondeur, les Rhizaria (appelés « Sarcodines » dans cette publication) représenteraient en moyenne 23% de la totalité du macro-zooplancton, soit le deuxième groupe planctonique en terme d'abondance. Cependant, les bénéfices de cette grande échelle spatiale sont nuancés par le manque de résolution taxonomique dans la définition des différentes catégories de Rhizaria (Figure 18), qui ne permet pas d'étudier en détail leurs distributions. Il est aussi fort probable que certaines catégories soient redondantes (par exemple RadioC et RadioCD qui sont probablement toutes deux des catégories rassemblant des Collodaires coloniaux), là où, à l'inverse, certaines catégories auraient pu être séparées en plusieurs nouveaux groupements (ex : RadioCS) afin d'améliorer la résolution taxonomique du jeu de données. À plusieurs reprises, les méthodes d'imagerie *in situ* ont donc montré leur efficacité dans l'observation et la quantification des Rhizaria, en particulier des Collodaires. Ces données sont encore trop parcellaires pour pouvoir réellement comprendre l'importance des Collodaires dans les écosystèmes marins. Afin de pouvoir quantifier spécifiquement l'abondance et la biomasse de ce groupe, il est nécessaire de pouvoir coupler une résolution taxonomique fine à une large échelle spatiale.

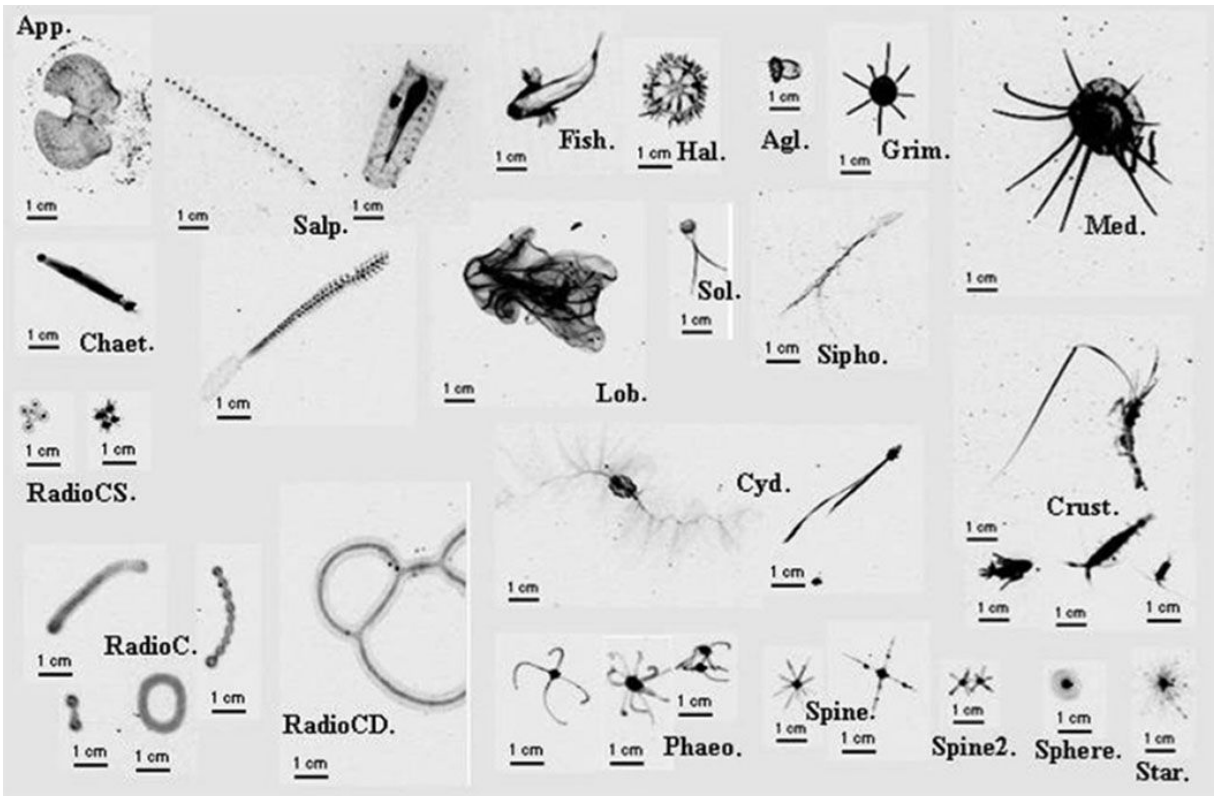

Figure 18 | Catégories d'images acquises par l'Underwater Video Profiler (Stemmann et al., 2008). Les Rhizaria regroupent huit groupes (RadioCS, RadioC, RadioCD, Phaeo, Spine, Spine2, Sphere et Star) définis sur la base de morphologie de l'organisme.

Objectifs de la thèse

1) Etablir un cadre de référence morpho-moléculaire pour l'ordre des Collodaires à partir d'une série de Collodaires (coloniaux et solitaires), collectés dans plusieurs régions océaniques, et à l'aide d'une double approche, intégrant des analyses morphologiques et des analyses phylogénétiques.

2) Une fois le cadre morpho-moléculaire établi, explorer la biodiversité environnementale des Collodaires à l'échelle globale à travers l'expédition *Tara* Océans. Etablir une biogéographie globale des différentes familles et clades de Collodaires, et identifier les relations entre les variations de diversité et les paramètres abiotiques.

3) Examiner les dynamiques saisonnières d'une communauté de Collodaires au cours du temps, ses variations de diversité ou ses interactions avec les autres groupes de Rhizaria, grâce à un suivi saisonnier de 18 mois réalisé dans la Baie de Villefranche-sur-Mer entre 2013 et 2014.

4) Quantifier les abondances et biomasses de ces différents groupes à l'aide de la technologie d'imagerie *in situ* Underwater Vision Profiler, et comparer leurs distributions verticales et horizontales. Etudier la biogéographie globale des Collodaires et des différents taxons composant le super-groupe des Rhizaria. Déterminer l'importance des Collodaires vis-à-vis des communautés zooplanctoniques à l'échelle des différentes régions biogéochimiques.

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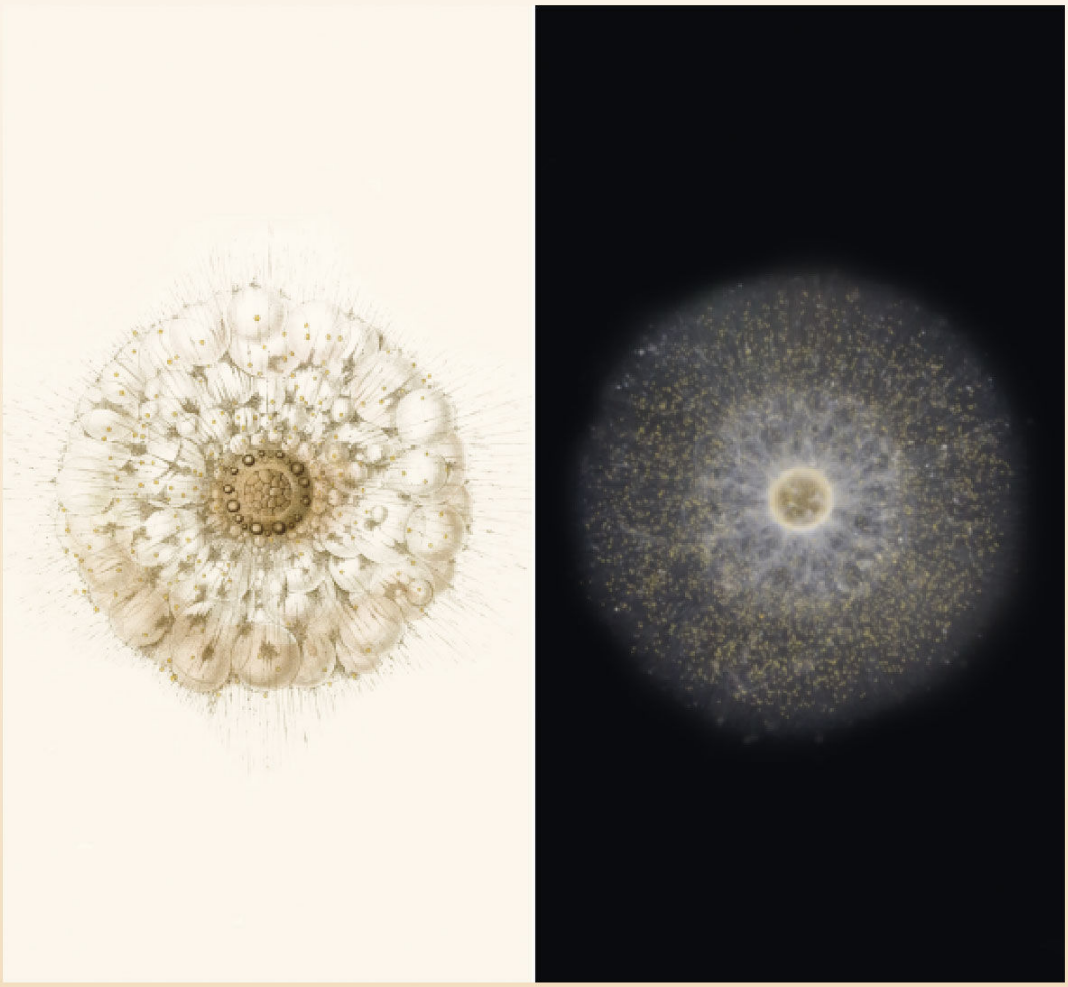

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CHAPITRE I

CLASSIFICATION OF THE MARINE PROTIST COLLODARIA

« Esteemed Sir (…) I already venerated the discoverer of the Thalassicollae and Collosphaerae as an especially outstanding member of the naturalists' reform, who at this time in England just as in Germany are fighting for the further development of the genealogical way of looking at nature, and not losing the philosophical point of view in specialised research. »

Ernst Haeckel to Thomas Henry Huxley (1862)

Towards an integrative morpho-molecular classification of the Collodaria (Polycystinea, Radiolaria)

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Abstract

Collodaria are ubiquitous and abundant marine radiolarian (Rhizaria) protists. They occur as either large colonies or solitary specimens, and, unlike most radiolarians, some taxa lack silicified structures. Collodarians are known to play an important role in oceanic food webs as both active predators and hosts of symbiotic microalgae, yet very little is known about their diversity and evolution. Taxonomic delineation of collodarians is challenging and only a few species have been genetically characterized. Here we investigated collodarian diversity using phylogenetic analyses of both nuclear small (18S) and large (28S) subunits of the ribosomal DNA, including 124 new sequences from 75 collodarians sampled worldwide. The resulting molecular phylogeny was compared to morphology-based classification. Our analyses distinguished the monophyletic clade of skeleton-less and spicule-bearing Sphaerozoidae from the sister clades Collosphaeridae (skeleton-bearing) and Collophidiidae (skeleton-less), while the Thalassicollidae was not retrieved as a monophyletic clade. Detailed morphological examination with electron microscopy combined with molecular analyses revealed many discrepancies, such as a mix between solitary and colonial species, co-existence of skeleton-less and skeleton-bearing specimens within the Collosphaeridae, as well as complex intraspecific variability in silicified structures. Such observations challenge a morphology-based classification and highlight the pertinence of an integrative taxonomic approach to study collodarian diversity.

Introduction

Radiolaria is a lineage of marine planktonic protists that appeared in the early Paleozoic and belongs to the supergroup Rhizaria (Adl et al. 2005; Nikolaev et al. 2004). Radiolarians are classified in five taxonomic orders, mainly distinguished by the morphology and composition of their mineral skeletons: strontium sulphate in Acantharia, silica in Taxopodida and in the polycystine Collodaria, Nassellaria and Spumellaria (Suzuki and Aita 2011). While many previous studies have investigated the past diversity and paleo-environmental signatures of radiolarians through their fossil record, less is known about the diversity and ecology of extant polycystines in marine ecosystems.

Collodarians are widespread in the oceans, exhibiting high abundances in calm and oligotrophic surface waters (Swanberg 1979). High densities of collodarian colonies have been reported in the Gulf of Aden (16,000 - 20,000 colonies per m^3; Khmeleva 1967) and in the North Pacific Ocean (up to 30 colonies per m^3; Dennett et al. 2002). *In situ* observations and culture experiments have described collodarians as active predators feeding on a broad range of prey (e.g. copepods, ciliates, phytoplankton or bacteria; Anderson 1978; Swanberg and Caron 1991), therefore contributing significantly to oceanic food webs. In addition to their heterotrophic behaviour, all known colonial collodarians harbour hundreds of endosymbiotic microalgae (Hollande and Enjumet 1953), in most cases the dinoflagellate *Brandtodinium nutricula* (Probert et al. 2014). This makes collodarians significant contributors to primary production in oligotrophic surface waters (Michaels et al. 1995; Swanberg and Harbison 1980). The mixotrophic behaviour of collodarians, coupled with their wide distribution and abundance, emphasizes the ecological and biogeochemical significance of these uncultivated protists in oceanic waters (Anderson 1983; Dennett et al. 2002; Michaels et al. 1995). However, because of inadequate sampling and preservation procedures, they have often been neglected in environmental surveys and their ecological importance has very likely been underestimated.

Most Collodaria form colonies comprising tens to hundreds of individual radiolarian cells (i.e. central capsules) embedded in a gelatinous matrix that ranges from a few millimetres up to 3 meters long (Swanberg 1979). Large solitary species (i.e. one single radiolarian cell) of several millimetres have also been described within the Collodaria. Some species build a shell-like skeleton around their central capsule while others have siliceous spicules, similar to those in sponges, in the matrix and some lack mineral structures altogether. Taxonomically, collodarians have been grouped into three families based essentially on the presence or absence of colonial forms. The family Thalassicollidae is composed exclusively of solitary species that are classified based on the position of alveoli surrounding the central capsule or on the structure of the spicules (Anderson et al. 2002). The Collosphaeridae contains only colonial skeleton-bearing collodarians. As the siliceous shells of collodarians are preserved in sediments over large geological periods of time, micropaleontologists have described living and fossil species according to different features of the skeleton (shape and size; number and size of pores; position, size and number of openings; Strelkov and Reshetnyak 1971). The family Sphaerozoidae includes skeleton-less and spicule-bearing colonial taxa. For the latter, spicule shape (size, number of radiate spines, presence of appendages) and positions are

taxonomically informative characters (Brandt 1885; Haeckel 1887; Popofsky 1920). For the skeleton-less collodarians, taxonomic identification is more complex and transmitted light microscopy provides only very limited morphological information for their identification (Brandt 1885; Haeckel 1887). Swanberg (1979) noticed that the shapes of the colonies were not species-specific, but highlighted a set of morphological features (e.g. distribution of alveoli, gelatinous textures) that could potentially help in their identification. Fine structural analysis with transmission electron microscopy later allowed the separation of two skeleton-less colonial genera, *Collophidium* and *Collozoum*, within the Sphaerozoidae based on the shape of their central capsules and the density of cytoplasmic vacuoles (Anderson et al. 1999).

In light of the difficulty of achieving accurate morphological identification in uncultured protists, phylogenetic analyses with ribosomal genes have been shown to be valuable tools not only to assess the diversity and evolutionary patterns among these organisms, but also to define new taxonomic frameworks and identify life stages (Bachy et al. 2012; Decelle et al. 2012, 2013). For collodarians, phylogenetic studies based on the 18S rRNA gene have demonstrated that they form a distinct monophyletic group, included in the paraphyletic Nassellaria, and do not belong to the order Spumellaria as previously suggested (de Wever et al. 2001; Krabberød et al. 2011; Kunitomo et al. 2006). At the family level, the Collosphaeridae, Sphaerozoidae and Thalassicollidae form separate monophyletic clades, but their phylogenetic relationships remain unclear (Kunitomo et al. 2006; Zettler et al. 1999). Currently, with only 6 reference sequences, the family Thalassicollidae (i.e. the solitary species) constitutes the earliest diverging clade in most phylogenies (Ishitani et al. 2012; Krabberød et al. 2011; Kunitomo et al. 2006; Polet et al. 2004; Yuasa et al. 2005). The phylogenetic position of the Thalassicollidae and analyses of their nuclei led to the hypothesis that solitary cells represent the ancestral form for Collodaria (Suzuki et al. 2009). Recently, Ishitani et al. (2012) proposed a new phylogenetic scheme for the Collodaria, with a clade composed of sequences affiliated to the genus *Collophidium* and the informal proposition (International Code of Zoological Nomenclature rules were not respected) of a fourth collodarian family, the Collophidae. Yet, with only two sequences and weak support values, the robustness of this family remains to be assessed.

With only a few reference ribosomal sequences (22 18S rDNA and 2 28S rDNA sequences) available in GenBank (as of July 2014) relationships between collodarian families, genera and species that typically lack informative morphological characters, are still poorly resolved. In addition to clarifying these relationships, sequencing morphologically identified specimens collected in the environment will contribute to the construction of a robust reference database of barcodes that will represent a powerful new tool to explore the ecology of collodarians at large spatio-temporal scales through metabarcoding approaches. The objective of the present study was to better understand the molecular diversity of Collodaria and the phylogenetic relationships between its taxa, by integrating morphological and molecular information.

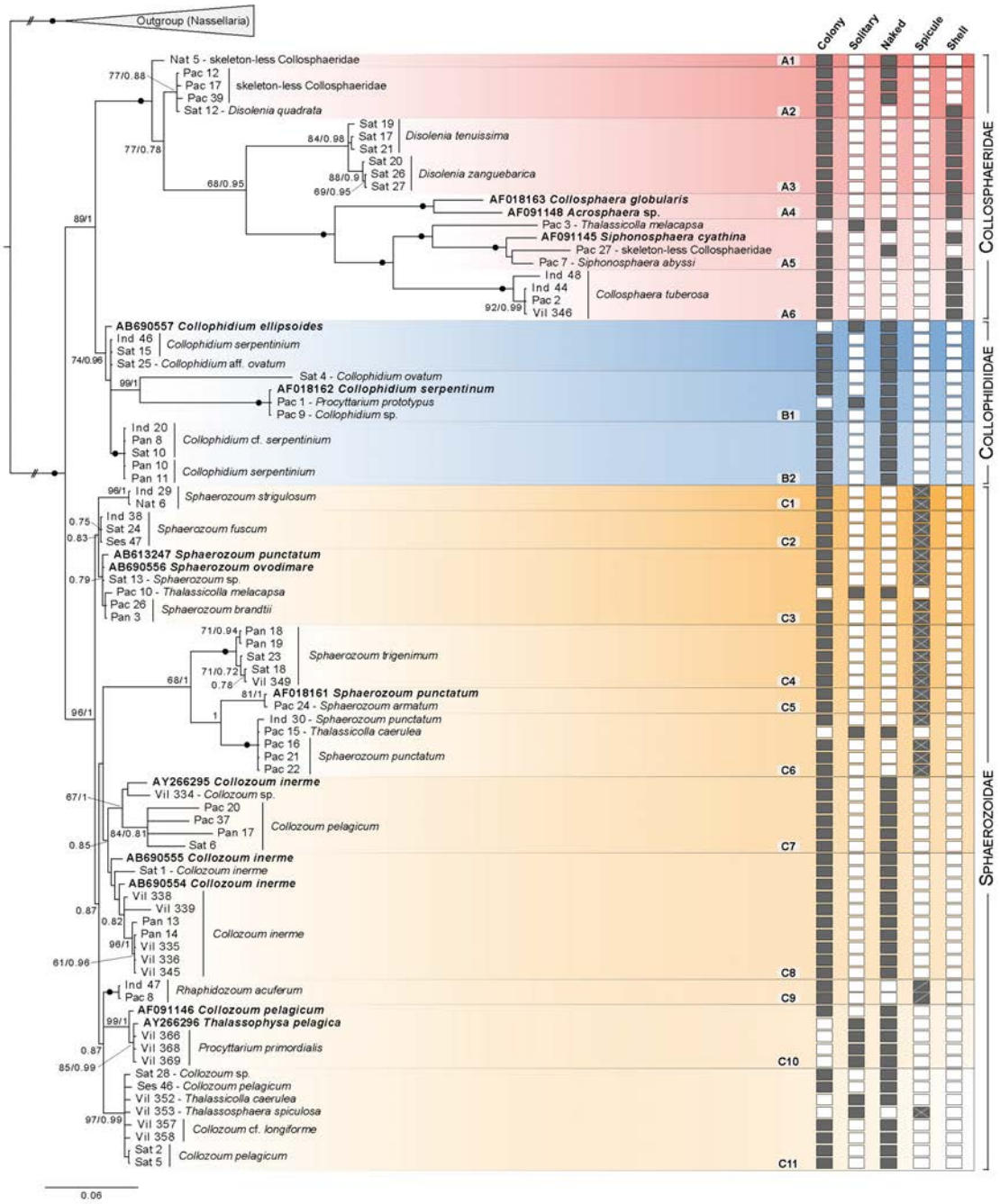

Figure 1. Molecular phylogeny of Collodaria inferred from the concatenated complete 18S and partial 28S (D1-D2 regions) rRNA genes (92 taxa and 1614 aligned positions). The tree was obtained by using a Bayesian method implemented using the GTR + Γ model of sequence evolution. PhyML bootstrap values (100 replicates) and MrBayes posterior probabilities are shown at the nodes when supports are higher than 60% and 0.7, respectively. Black circles indicate bootstrap support and posterior probabilities of 100% and 1.00, respectively. The three main clades, defined based on statistical support and morphological criteria, highlight the Collosphaeridae (A), Collophidiidae (B), Sphaerozoidae (C) families and their respective sub-clades. Morphological criteria are identified on the right for each specimen (dark grey: presence; white: absence): colonial or solitary, naked (lacking any silicified structure), spicule-bearing (one bar: simple spicules; two bars: radiate spicules) or skeleton-bearing. Reference sequences from GenBank are shown in bold. Six Nassellaria sequences were assembled as out-group (AB430759, AF382824, DQ386169, FJ032682, FJ032683, HQ651779). Branches with a double barred symbol are reduced to half-length for clarity.

Results

Molecular Phylogenies of Collodarian Families

A total of 75 collodarian specimens encompassing the four families, Sphaerozoidae, Collosphaeridae, Collophidiidae (formally described herein from the previously proposed Collophidae) and Thalassicollidae, were isolated from worldwide locations. All but nine of these specimens were colonial (Supplementary Material Fig. S1 and Table S1). From these specimens, 62 partial 18S rDNA and 62 partial 28S rDNA (D1-D2 regions) sequences were obtained (Supplementary Material Table S1). Phylogenetic analyses based on the concatenated dataset revealed 3 distinct clades, of which clades A and B, consisting of 18 and 11 sequences respectively, were the most closely related (Fig. 1). The third clade (clade C) contained most of the sequences (n = 46) and was basal to the other clades. Morphological identification of the analysed individuals showed that these monophyletic clades corresponded to three collodarian families: all skeleton-bearing (i.e. Collosphaeridae) occurred in clade A, the skeleton-less Collophidiidae grouped within clade B, while members of the Sphaerozoidae formed clade C. The fourth family of solitary Thalassicollidae was not retrieved as a monophyletic clade.

Within Collosphaeridae (Fig. 2), 18 novel and 3 previously available sequences revealed 6 distinct clades (A1 to A6) supported with moderate to high support values. Maximum values of ML bootstrap (BS) and posterior probability (PP) showed strong phylogenetic relationships between clades A4, A5 and A6, while weak support values suggested uncertain phylogenetic placement of clades A1, A2 and A3. The family Collophidiidae gathered 11 new and two reference sequences. Two clades (B1 and B2) contained 9 of the sequences whereas the other sequences could not be assigned to a specific clade. Both clades were supported with high support values but the phylogenetic relationships between them were not resolved. The Sphaerozoidae contained 46 new and 8 publicly available sequences distributed in 11 clades (C1 to C11). Most of the clades were highly supported except for clades C2, C3 and C7 that had weak support, and clade C8 that was not supported at all. Phylogenetic relationships between these clades remain unclear.

Integrative Taxonomy of Collodaria

Based on morphological observations performed with light microscopy (LM) and Scanning Electron Microscopy (SEM) images, the Collosphaeridae mainly included skeleton-bearing specimens easily assigned to different genera according to the overall morphology of their shells: *Disolenia*, *Collosphaera* and *Siphonosphaera* (Figs 1 and 2). The *Disolenia* species had a polygonal shell with large irregular polygonal openings, 2 to 9 in number (Fig. 2A-G). All *Collosphaera* species had a crumpled sphere-like shell with polygonal to rounded pores (Fig. 2H, I). *Siphonosphaera* shells were mostly spherical with characteristic tubular projections pointing outwards (Fig. 2J). The molecular classification broadly matched morphological identifications, these three genera clearly belonging to different clades in the phylogenetic analyses (Fig. 1). However, these analyses also highlighted several discrepancies between molecular and morphology-based classifications.

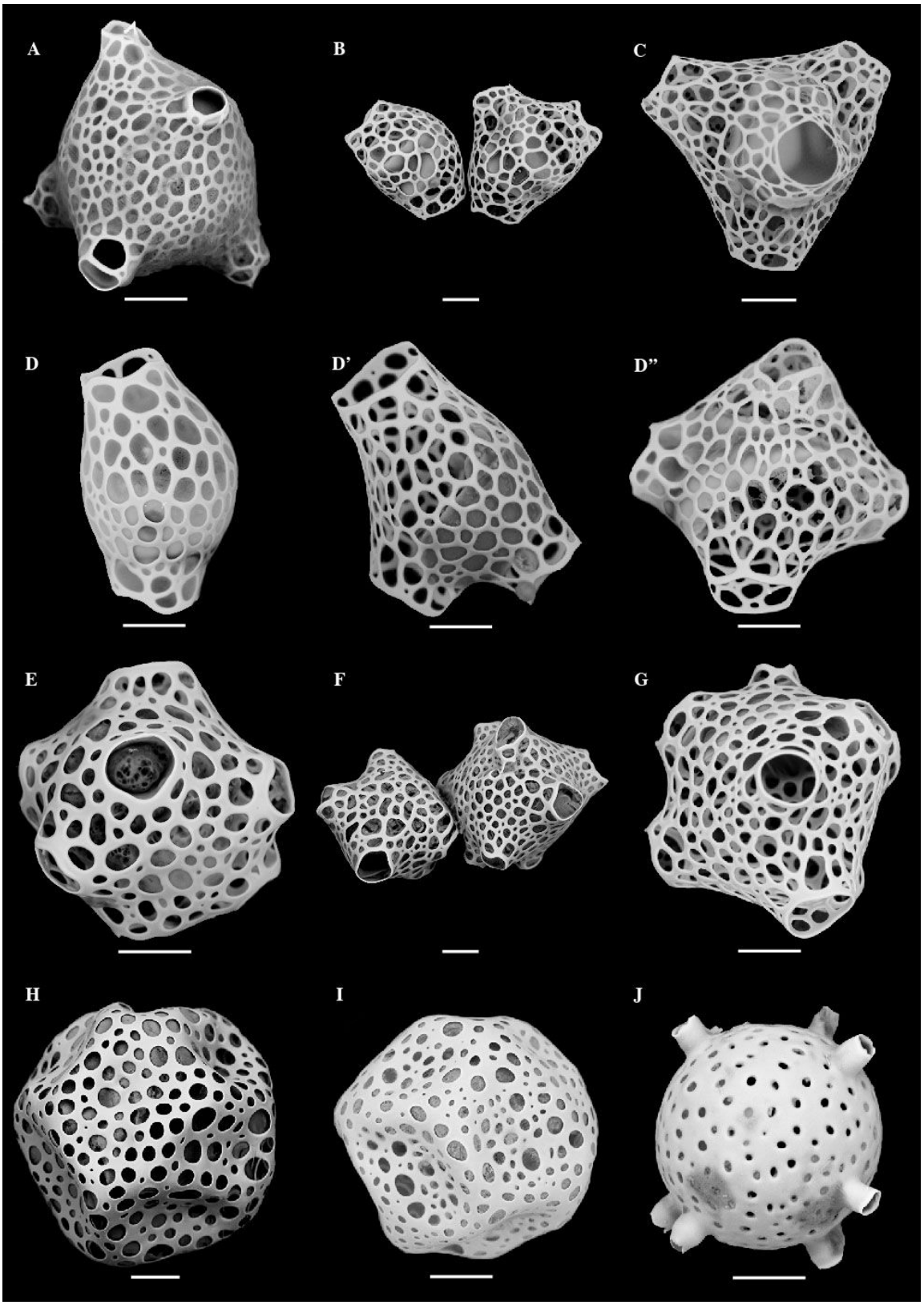

Figure 2. Scanning Electron Microscopy images of Collosphaeridae skeletons. All of these images were obtained from the exact same specimens used for the molecular phylogenetic analysis in this study. (**A**) *Disolenia quadrata* (Sat 12). (**B**) *Disolenia tenuissima* (Sat 17). (**C**) *D. tenuissima* (Sat 19). (**D-D"**) *D. tenuissima* (Sat 21). (**E**) *Disolenia zanguebarica* (Sat 20). (**F**) *D. zanguebarica* (Sat 26). (**G**) *D. zanguebarica* (Sat 27). (**H**) *Collosphaera tuberosa* (Ind 44). (**I**) *C. tuberosa* (Ind 48). (**J**) *Siphonosphaera abyssi* (Pac 7); scale bars = 30 µm.

In 5 of the colonial specimens from clades A1, A2 and A5, no shells were observed in either LM or SEM images (Supplementary Material Fig. S1). Specifically, in clades A2 and A5 these skeleton-less forms were mixed with skeleton-bearing specimens and two of them (Pac 12 and Pac 17) were even genetically similar to one skeleton-bearing species (Sat 12). For some skeleton-bearing specimens, several shell morphotypes were found among genetically identical specimens or even within a single specimen. For instance, in specimens Sat 17 (Fig. 2B) and Sat 21 (Fig. 2D-D"), both identified as *Disolenia tenuissima*, two and three shell morphotypes coexisted, respectively. Finally, one specimen (Pac 3), a solitary Thalassicollidae morphologically identified as *Thalassicolla melacapsa*, was genetically placed among the 17 other Collosphaeridae specimens, which were exclusively colonial specimens.

In Collophidiidae (Fig. 1), all 10 colonial specimens were morphologically identified as belonging to the genus *Collophidium* (Supplementary Material Fig. S1). Clade B1 included an assemblage of different morpho-species while clade B2 was only composed of a single morphologically identified species (*C. serpentinum*). As for the Collosphaeridae, colonial and solitary specimens were mixed within a single clade. For instance, clade B1 included the solitary *Procyttarium prototypus*, that formally belong to the Thalassicollidae, and which was genetically identical to two colonial *Collophidium*. Regardless of this mix between colonial and solitary species, the Collophidiidae contained only naked specimens.

Seven genera from both solitary and colonial forms were represented in the Sphaerozoidae: the colonial *Collozoum, Rhaphidozoum* and *Sphaerozoum*, and the solitary *Procyttarium*, *Thalassicolla*, *Thalassosphaera* and *Thalassophysa* (Fig. 1 and Supplementary Material Fig. S1). The skeleton-less genus *Collozoum* was highly polyphyletic as it was represented in clades C7, C8, C10 and C11. Although it was not supported, Clade C6 consistently gathered only the species *Collozoum inerme*. The clade was composed of a core of 5 sequences, supported with high support values, and 5 other sequences having a strong affinity for this core group. Several different morphologies (cell size, shape and organisation within the colony) were distinguished between clades, but affiliation to known species was unclear. The specimens forming clades C1-C6 and C9 were mainly spicule-bearing Sphaerozoidae (Fig. 3). Cell shrinkage caused by ethanol preservation disrupted the arrangement of spicules inside the colony leading to challenging taxonomical identification based on LM observation (Supplementary Material Fig. S1). The two C9 specimens representing the species *Rhaphidozoum acuferum* had clearly identifiable needle-like spicules (Fig. 3A, B), but the relation between clade C9 and other spicule-bearing clades was unclear. Clades C1-C6 contained the other spicule-bearing specimens, all belonging to the genus *Sphaerozoum*, recognizable by their double tri-radiate spicules (Fig. 3C-K). Based on SEM images, detailed examination of all specimens in clade C6 (identified as *S. punctatum*) revealed numerous small spicules and a few larger ones (Fig. 3H, H'). Differences in spicule structure was also observed in other *Sphaerozoum* species and ranged from a more complex spinose pattern to the appearance of a fourth radiate axis (Supplementary Material Fig S2). As for the two other families, while 39 specimens of the Sphaerozoidae were colonial, clades C3, C6, C10 and C11 also included solitary species from the Thalassicollidae (Fig. 1 and Supplementary Material Fig. S1).

Environmental Diversity of Collodaria

To evaluate the robustness of the coverage of our taxonomic sampling, the 92 environmental sequences affiliated to Collodaria and currently available in GenBank (July 2014) (Supplementary Material Table S2) were placed in the phylogenetic tree composed of our reference sequences (Fig. 4). The majority of environmental sequences (91%) grouped within clade B (Collophidiidae). More detailed placements mapped 1 and 2 sequences into clades B1 and B2, respectively. A majority of sequences (81) could not be precisely assigned to any known clade of Collophidiidae. Collosphaeridae represented 6.5% of the environmental sequences (3 in clade A5, 1 in A1, and 2 not precisely assigned). The last family, Sphaerozoidae, only included one environmental sequence affiliated to clade C8.

Discussion

Morpho-molecular Classification and Evolution of Collodaria

The morpho-molecular approach used in this study provides new insights into collodarian diversity and challenges the traditional classification and hypotheses on the evolutionary history of the group. From our phylogenetic analysis we recovered the monophyly of three of the four collodarian families (Collosphaeridae, Collophidiidae and Sphaerozoidae), while members of the polyphyletic Thalassicollidae were scattered throughout the tree (Fig. 1). The Thalassicollidae was historically created to group solitary specimens, but the co-occurrence we observed between solitary taxa and colonial species highlights a major discrepancy between molecular and morphological classification, and questions the validity of this character as a reliable taxonomic marker. Previous studies supported a phylogenetic separation of solitary and colonial species (Polet et al. 2004; Zettler et al. 1999), yet none of our solitary specimens, nor any environmental sequences, clustered with the previously described Thalassicollidae clade (data not shown). In addition, careful analysis of the DNA sequences of the Thalassicollidae reveals that the distinction and monophyly of this clade was essentially based on large ambiguous regions in the 18S rRNA gene (see Methods section). Although we cannot precisely determine their origin, these ambiguous regions may correspond to pseudo-genes, which have been identified in some marine protists and are generally highly divergent from native ribosomal sequences (Santos et al. 2003; Thornhill et al. 2007). The inconsistency of the Thalassicollidae family in our analyses questions its basal position in the Collodaria and highlights the need to reconsider the hypothetical evolutionary history stating that Collodaria evolved from solitary Thalassicollidae to colonial Sphaerozoidae and then to skeleton-bearing colonial Collosphaeridae (Suzuki et al. 2009).

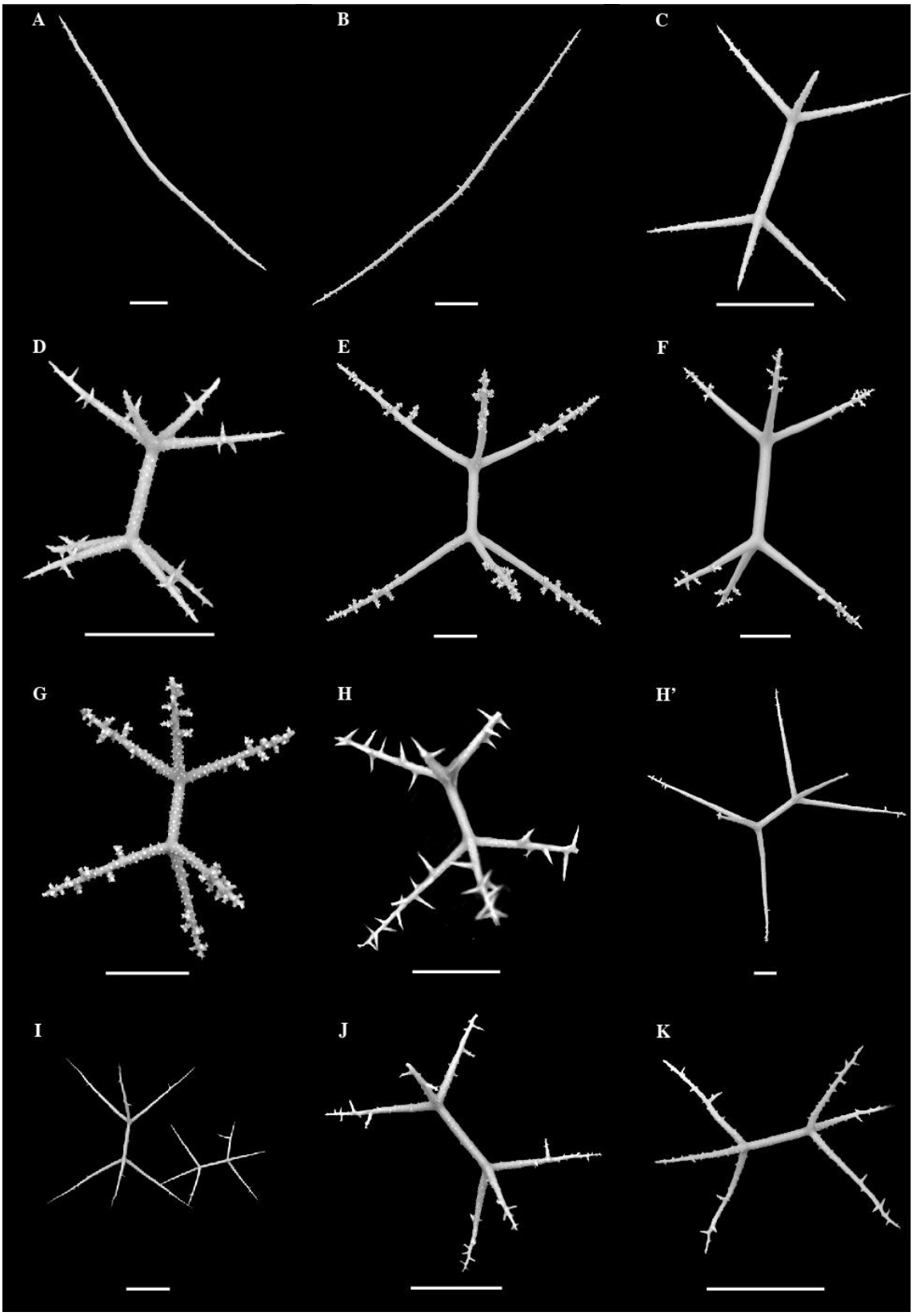

Figure 3. Scanning Electron Microscopy images of Sphaerozoidae spicules. All of these images were obtained from the exactly same specimens used for the molecular phylogenetic analysis in this study. (A) *Rhaphidozoum acuferum* (Ind 47). (B) *R. acuferum* (Pac 8). (C) *Sphaerozoum fuscum* (Sat 24). (D) *Sphaerozoum strigulosum* (Nat 6). (E) *Sphaerozoum brandtii* (Pac 26). (F) *S. brandtii* (Pan 3). (G) *Sphaerozoum armatum* (Pac 24). (H-H') *Sphaerozoum punctatum* (Pac 21). (I) *Sphaerozoum trigeminum* (Pan 19). (J) *S. trigeminum* (Sat 23). (K) *S. trigeminum* (Sat 18); scale bars = 20 μm.

In our analyses, colonial and solitary specimens were genetically close or even identical in each of the three families, suggesting that they represent the same taxonomic entity and could be two distinct phases of the same life cycle. Knowledge about the life cycle in Collodaria is very limited and relies mainly on old studies. Several solitary forms have already been associated to colonial species (e.g. *Thalassophysa sanguinolenta* and *Collozoum pelagicum*) (Brandt 1902). This association was later supported with phylogenetic analyses of both solitary and colonial forms (Polet et al. 2004; Zettler et al. 1999). Investigating reproductive mechanisms in Collodaria, Hollande and Enjumet (1953) pointed out the ability of solitary specimens to create "proto-colonies" through the simple budding of the cell. The release of flagellate swarmers was also observed in both colonial and solitary collodarian species (Anderson 1976, 1978; Hollande and Enjumet 1953), but the fate of these swarmers remains unknown to date.

The phylogenetic position of the Collophidiidae (clade B) as a sister-clade of the Collosphaeridae (clade A) is congruent with a previous phylogenetic study (Ishitani et al. 2012). The phylogenetic analyses clearly discriminate the Collophidiidae from the other skeleton-less family (i.e. Sphaerozoidae) as shown in Figure 1. The colonial genus *Collophidium*, originally included in the Sphaerozoidae, showed major ultrastructural and molecular differences with the genus *Collozoum* (Anderson et al. 1999; Zettler et al. 1999). Molecular divergence between *Collophidium* and the Sphaerozoidae was later suggested (Ishitani et al. 2012). Here, following the rule of the International Code of Zoological Nomenclature (ICZN 1999), we formally propose the erection of a new family: Collophidiidae Biard et Suzuki fam. nov. (with *Collophidium* as the type genus, *Collophidium serpentinum* as the type species; see taxonomic appendix below).

Collodarian shells have been suggested to have originated from the fusion of spicules in a spicule-bearing ancestor (Anderson and Swanberg 1981; Strelkov and Reshetnyak 1971). This hypothesis was later rejected because of the absence of congruent fossil records to link Sphaerozoidae and Collosphaeridae (Bjørklund and Goll 1979). In our morpho-molecular analyses, there is a clear separation between all skeleton-bearing (Collosphaeridae) and all spicule-bearing specimens (Sphaerozoidae) (Fig. 1). This suggests that skeleton-bearing and spicule-bearing collodarians have co-diversified and that the skeleton is not a derived character. Our results also rule out the possibility of the presence of both spicules and a shell during the life cycle of collodarians.

The Need for an Integrative Taxonomic Approach

In the present analyses, the monophyletic Sphaerozoidae (Fig. 1) contains three genera, *Collozoum*, *Sphaerozoum* and *Rhaphidozoum*, which is congruent with the traditional taxonomical scheme (Anderson 1976; Strelkov and Reshetnyak 1971). The high polyphyly of the genus *Collozoum*, which is distributed in clades C7, C8, C10 and C11, suggests that specific morphological characters need to be revised (Fig. 1). The name *Collozoum inerme* has often been used as generic species name for nearly all skeleton-less collodarians, although in Haeckel's descriptions (1862) it is characterized by the presence of an oil-like droplet in the central capsule of the cell. In our specimens, such a structure was only found for members

of clade C8, subsequently identified as *Collozoum inerme*. Specimens found in clade C7 and C10 presented distinct morphological features compared to clade C8, having more algal symbionts, a transparent layer around the endoplasm and spherical collodarian cells. Whether these morphological features are taxonomically reliable characters, implying that they are "fixed" during the life cycle and in different environmental conditions, remains to be determined.

In the Collosphaeridae, the co-existence of both skeleton-less and skeleton-bearing specimens occurred in two clades (Fig. 1). We were unable to identify the skeleton-less stages following the taxonomical scheme for Collosphaeridae. Haeckel (1862) first pointed out the absence of a skeleton in many colonies and the existence of skeleton-less cells during the early developmental stages of Collosphaeridae, which was later confirmed through culture attempts (Anderson and Gupta 1998; Anderson and Swanberg 1981). Since the traditional taxonomical scheme to identify Collosphaeridae is based on skeleton morphology, Anderson and Swanberg (1981) mentioned the impossibility to identify such cells in early stages of skeletal development. The existence of skeleton-less cells within a skeleton-bearing clade challenges morphological identification and emphasizes the requirement for novel morphological characters as well as molecular tools for accurate identification of different life stages.

In both Collosphaeridae and Sphaerozoidae, detailed morphological examinations revealed intraspecific variability in silicified structures (i.e. shell and spicules) (Figs 2 and 3, and Supplementary Material Fig. S2). Several shell morphotypes were found among genetically identical specimens or even within a single specimen (i.e. *Disolenia tenuissima*) (Fig. 2B-D"). Originally described with one or two, and occasionally three large openings (Hilmers 1906), *D. tenuissima* exhibited variations in shell pores that have been previously reported for other skeleton-bearing collodarians, and likely explained by the ontogeny of the silicified structure (Anderson and Swanberg 1981). These observations challenge Haeckel's classification based on shell features, as the existence of different morphotypes leads to uncertain taxonomic identification. As for the Collosphaeridae, several spicule-bearing Sphaerozoidae exhibited intraspecific variability of their spicules. The morphological taxonomic criteria classically used to discriminate between *Sphaerozoum* species include spinose patterns, arrangement and number of spicules, and the development state of an oil-like droplet inside each cell (Anderson 1983). The co-existence of small and rare larger spicules has already been reported within colonies of *S. punctatum* and *S. fuscum* (Brandt 1881; Popofsky 1920). Whereas differences in spicule thickness can be explained by ontogeny (Brandt 1881), the range of variability in spicules encountered (different sizes or shapes) suggests more complex processes. Fusion between colonies, likely of the same species, is regularly observed (Hollande and Enjumet 1953; pers. observ.), but it is not clear whether these physical associations are temporary during the life cycle, or represent reproductive mechanisms, predatory relationships, or simply an experimental artefact (Hollande and Enjumet 1953; Huth 1913; Swanberg 1979). Therefore, we cannot exclude the possibility that a mix of shells or spicules within one specimen could be the consequence of such fusion, potentially blurring morphology-based taxonomic identifications.

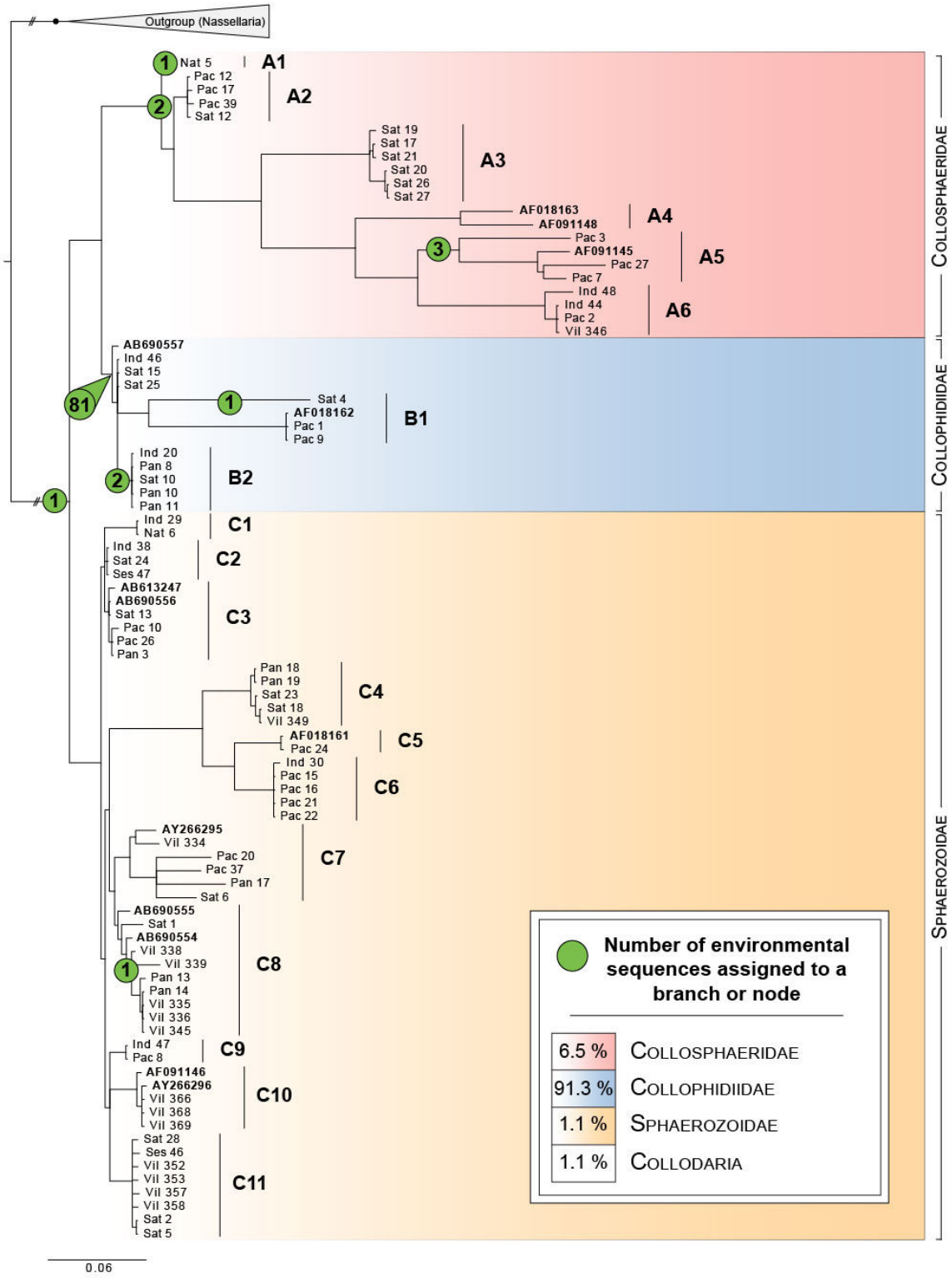

Figure 4. pplacer phylogenetic placement of 92 environmental sequences onto a concatenated phylogenetic tree of Collodaria (complete 18S + partial 28S rRNA genes). Number of sequences affiliated to a clade (node) or sub-clade (branch) is shown inside green circles.

Insights into the Diversity of Collodaria in the Environment

The present morpho-molecular framework for Collodaria allowed a more accurate taxonomic placement of sequences derived from environmental molecular diversity surveys available in public databases (Fig. 4 and Supplementary Material Table S2). Environmental sequences belonged to the three families highlighted previously (i.e. Collosphaeridae, Collophidiidae, Sphaerozoidae). Whereas most of the environmental sequences belonged to the Collophidiidae, the most represented family in our study was the Sphaerozoidae. This discrepancy could be explained by the fact that all specimens used in the present study were collected in surface waters and that most environmental sequences come from the entire water column in the Cariaco Basin, a very unusual ecosystem, or from deep samples (Edgcomb et al. 2011; Jungbluth et al. 2013; Sauvadet et al. 2010; Supplementary Material Table S2). Consequently, our sampling strategy could have introduced a bias against Collophidiidae, or other specific taxa, if they have marked ecological preferences impacting their distribution. A recent global environmental diversity survey based on metabarcodes of the V9 region of the 18S rRNA gene highlighted very high abundance, diversity and distribution of collodarian reads in the world oceans but could not identify the distribution of specific taxa (de Vargas et al. in press). Together with appropriate contextual data, the present morpho-molecular database sets the basis for a better understanding of the phylogeography and ecology of individual collodarian taxa.

Taxonomic Appendix

Family Collophidiidae Biard et Suzuki n. fam.

Type genus: *Collophidium* Haeckel, 1887 (type species: *Collozoum* (*Collophidium*) *serpentinum* Haeckel, 1887, designated by Campbell, 1954; raised to genus level by Anderson et al. 1999).

Synonymy: Collophidae in Ishitani et al. (2012) [unavailable name; see remarks].

Definition: Colonial Collodaria with a variable overall appearance of elongate, cylindrical, spherical form. Each colony comprises delicate gelatinous material encompassing scattered algal symbionts and string-like aggregations. A string-like aggregation consists of tens to a hundred collodarian cells in firm gelatinous material. This string-like aggregation ranges from several millimetres to several centimetres in length and approximate 0.5 mm in width. Algal symbionts are always distributed throughout fragile gelatinous material but not in string-like aggregations. The number of algal symbionts in a colony is variable. Each collodarian cell displays opaque inner protoplasm with surrounding transparent protoplasm. Both opaque inner and surrounding transparent protoplasm are defined as the endocapsulum. The outer transparent protoplasm consists of a layer of vacuoles whereas the inner opaque protoplasm contains organelles such as Golgi, nucleus, and mitochondria.

Remarks: A molecular phylogenetic study performed by Ishitani et al. (2012) informally reported the separation of *Collophidium* at the family level. Here we formally established the family Collophidiidae following the rules of the International Code of Zoological Nomenclature (ICZN 1999).

Methods

Sample collection: Plankton samples were collected in the bay of Villefranche-sur-Mer (France, 43°41′10″ N, 7°18′50″ E) using a Regent net (680 µm mesh size), off Sesoko Island, Okinawa (Japan, 26°37′20″ N, 127°52′15″ E) by net tows (20 and 150 µm mesh sizes) and during the TARA Oceans expedition using a Bongo net (180 µm mesh size) or a hand net (Supplementary Material Table S1). Specimens were immediately handpicked individually from the plankton samples with autoclaved micropipettes, transferred into clean beakers filled with 0.2-1 µm filtered seawater and incubated at 18 °C. After 3-4 hours the specimens had self-cleaned and were transferred individually into clean containers filled with 0.2 µm filtered sea-water. Images were then taken under a binocular microscope or an inverted microscope for higher magnification pictures. Each specimen was finally rinsed three times in 0.2 µm filtered seawater to avoid contamination and transferred into 1.5 ml Eppendorf tubes containing 50-150 µl absolute ethanol or 50 µl of Guanidine Isothiocyanate (GITC). Isolated specimens were stored at -20 °C until DNA extraction. Each isolated specimen was identified based on morphological criteria and images were compared to the original monographs. Tentative affiliations to known species were carried out following the original descriptions of Brandt (1885), Haeckel (1887), Popofsky (1920), Strelkov and Reshetnyak (1971). Detailed information related to each of the samples used in this study can be found in the RENKAN database at http://renkan.sb-roscoff.fr.

DNA extraction, amplification and sequencing: Genomic DNA from ethanol fixed specimens was extracted using the MasterPure Complete DNA Purification kit (Epicentre) following the manufacturers instructions. For GITC fixed specimens, extraction was performed as described in Decelle et al. (2012). Each specimen was extracted individually in an autoclaved microtube to avoid cross-contamination between samples. Pellet debris from DNA extraction were eluted in milliQ water to recover collodarian skeletons and spicules, and subsequently stored at -20 °C. Complete Small SubUnit (18S-SSU) and partial (D1 - D2 regions) Large SubUnit (28S-LSU) of the rDNA were amplified using Collodaria-specific primers (Table 1). The sets of primers SA/S81col and S32col/V9R amplify the first 1500 positions and last 1200 positions of the complete 18S rRNA gene, respectively. The set of primers 28S Col-F/ITSa3 Col amplifies 640 positions of the D1-D2 regions of the 28S rRNA gene. PCR reaction mix contained 8.75 µl of sterile water, 1 µl of each primer (10 µM concentration), 0.75 µl dimethyl sulfoxide (DMSO), 12.5 µl of the Phusion® High-Fidelity PCR Master Mix (Finnzymes) and 1 µl of DNA template, in a 25 µl final volume. PCR amplification conditions were: 30 s initial denaturation at 98 °C followed by 35 cycles of 10 s denaturation at 98 °C, 30 s of annealing at 53-58 °C according to the primers set, 72 °C for 30 s elongation and a 10 min final elongation step at 72 °C. All PCR amplifications were conducted in a PCR workstation, using autoclaved microtubes and molecular grade water. Amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide. Successfully amplified products were sent for sequencing at the GENOSCOPE (France). Sequencing was also performed locally on an ABI-PRISM 3100 Genetic Analyzer using the ABI BigDye Terminator v3.1 kit (Applied Biosystems) after a step of purification using the

NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel) following the manufacturer's instructions.

Table 1. Oligonucleotide primers used for PCR amplifications and sequencing.

GENE	PRIMER	Specificity	SEQUENCE 5'-3'	TYPE	REFERENCE
18S (1st part)	SA	Eukaryote	AAC CTG GTT GAT CCT GCC AGT	Forward	Medlin et al. 1988
	S81 Col	Collodaria	ATC ACA GAC CTG TYA TTG CWA	Reverse	This study
18S (2nd part)	S32 Col	Collodaria	TAT GCT AAC RWT GYT GCA	Forward	This study
	V9R	Eukaryote	CCT TCY GCA GGT TCA CCT AC	Reverse	Romac (unpub.)
28S	28S Col-F	Collodaria	TGG ACT TTC TAA GTA ATG GCG	Forward	This study
	ITSa3 Col	Collodaria	CAC CAT CTT TCG GGT CCC AGT	Reverse	This study

Phylogenetic analyses: Sequence quality was carefully checked on the chromatograms using the 4Peaks software. The forward and reverse fragments were then assembled using Seaview version 4.4.2. (Gouy et al. 2010) and the presence of chimeras was verified using the online Key DNA Tools (http://keydnatools.com/). All valid sequences were then compared to reference sequences using the BLAST tool and similar sequences identified on GenBank were integrated into our 18S and 28S databases. Careful examination of the Thalassicollidae 18S rDNA reference sequences AF057741, AF057742, AF057743, AF057744, AF018160 and AY266297 revealed two ambiguous regions in all of them, located at positions 59-274 base pairs (bp) and 599-819bp, totally different (0% identity) from all other collodarian sequences (except themselves) and from any other sequence published in GenBank. When removing these ambiguous regions from our alignment, these 5 sequences formed a clade where none of our single-cell sequences, neither any environmental sequences were retrieved (data not shown). These ambiguous 18S rDNA sequences were therefore removed from the subsequent phylogenetic analyses. According to previous studies, six nassellarian sequences (AB430759, AF382824, DQ386169, FJ032682, FJ032683, HQ651779) were used as outgroup (Krabberød et al. 2011). For each dataset, 18S (78 taxa; 1744 positions) and 28S (75 taxa; 666 positions), sequences were aligned using MAFFT version 7.213 (Kuraku et al. 2013) and ambiguous positions were manually removed. After the removal of ambiguous positions, 18S and 28S dataset included 1311 and 307 positions, respectively. Prior to phylogenetic analyses, the Perl script MrAIC 1.4.3 (Nylander 2004) in combination with PHYML v2.4.4 (Guindon and Gascuel 2003) was used to choose the best model of sequence evolution by the Akaike Information Criterion (AIC). Phylogenetic analyses were performed (as described below) independently on each gene marker (18S and 28S rDNA) and obtained topologies were compared in order to make sure that no paralogy issues exist and the two genes can be concatenated. As suggested in Decelle et al. 2012 or Krabberød et al. 2011, concatenation of both 18S and 28S rRNA genes in radiolarians phylogenies increase the phylogenetic resolution. Both 18S rDNA and 28S rDNA alignments were therefore concatenated using Sequence Matrix version 1.7.8. (Vaidya et al. 2011). Applying the obtained settings (GTR + Γ model) a Bayesian Inference (BI) method and a Maximum Likelihood (ML) method (Felsenstein 1981) were used to infer phylogeny. With the MrBayes program (Huelsenbeck and Ronquist 2001), two independent analyses were performed at the same time with four simultaneous chains (one cold and three heated) ran for 10 million generations, and sampled

every 1000 generations. After discarded 2000 of the initial trees as burn-in, the consensus tree with the corresponding posterior probabilities was calculated for each data set. The ML method was implemented with the PhyML v3.0 software (Guindon et al. 2010) and the reliability of internal branches was assessed using the bootstrap method with 100 replicates (Felsenstein 1985). In parallel, another Bayesian analysis was performed using the PhyloBayes software (Lartillot et al. 2009), in order to test the CAT-GTR model of sequences evolution (Lartillot and Philippe 2004), allowing heterogeneity across sites and which has been shown to potentially improve phylogenetic inference (Tsagkogeorga et al. 2009). This latter approach did not significantly improve the final topology and phylogenetic analyses inferred using the GTR + Γ model of sequences evolution were used. Bootstrap supports (BS) and posterior probabilities (PP) were associated to each node in the Bayesian topology. Final tree was visualized and edited in Fig Tree v 1.4.0. (Rambaut 2010). All sequences generated in the present study have been deposited in the GenBank database under accession numbers KR058196-KR058319. All alignments used in this study can be found online in the RENKAN database at http://renkan.sb-roscoff.fr.

Environmental diversity of Collodaria: For each family, closely related environmental 18S rDNA sequences available in GenBank (July 2014) were selected using BLAST in order to infer the environmental genetic diversity of Collodaria (Supplementary Material Table S2). Positions of these environmental sequences in our reference phylogenetic tree were determined using the pplacer software (Matsen et al. 2010) as earlier described in Decelle et al. (2012).

Scanning electron microscopy (SEM) observation: Eluted pellet debris containing collodarian skeletons or spicules recovered after DNA extractions were vortex mixed and sorted using an inverted microscope. Several skeletons and spicules were handpicked for each specimen and finally transferred into 0.2 ml Eppendorf tubes containing 50 µl hydrogen peroxide. Each tube was heated at 70 °C for 10 min to remove residual organic matter. Several clean skeletons or spicules were then handpicked and placed on 0.2 µm pore-size polycarbonate membrane filters, dried and stuck on SEM pin stub mounts. Filters were imaged with an FEI Phenom tabletop SEM (FEI Technologies).

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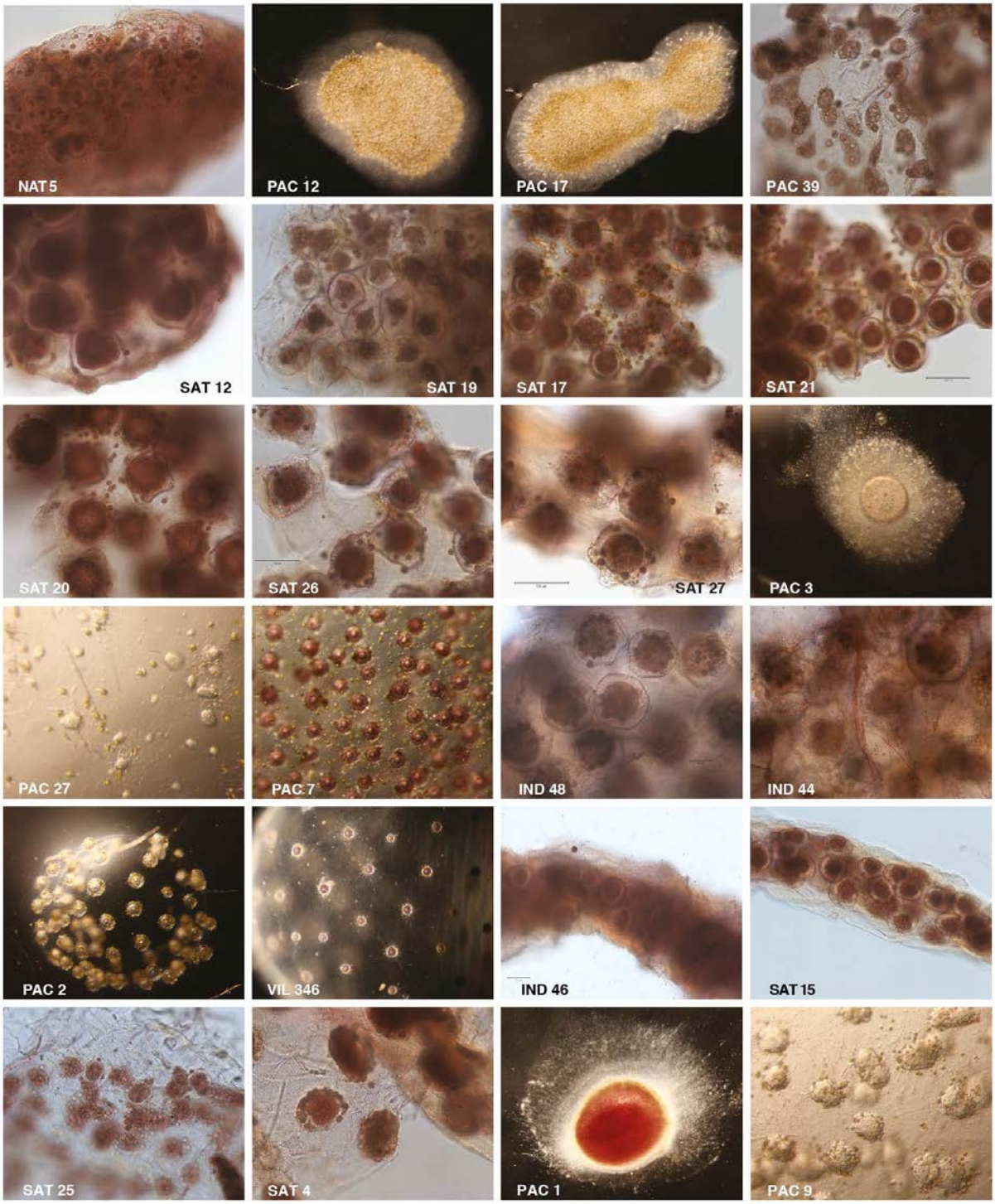
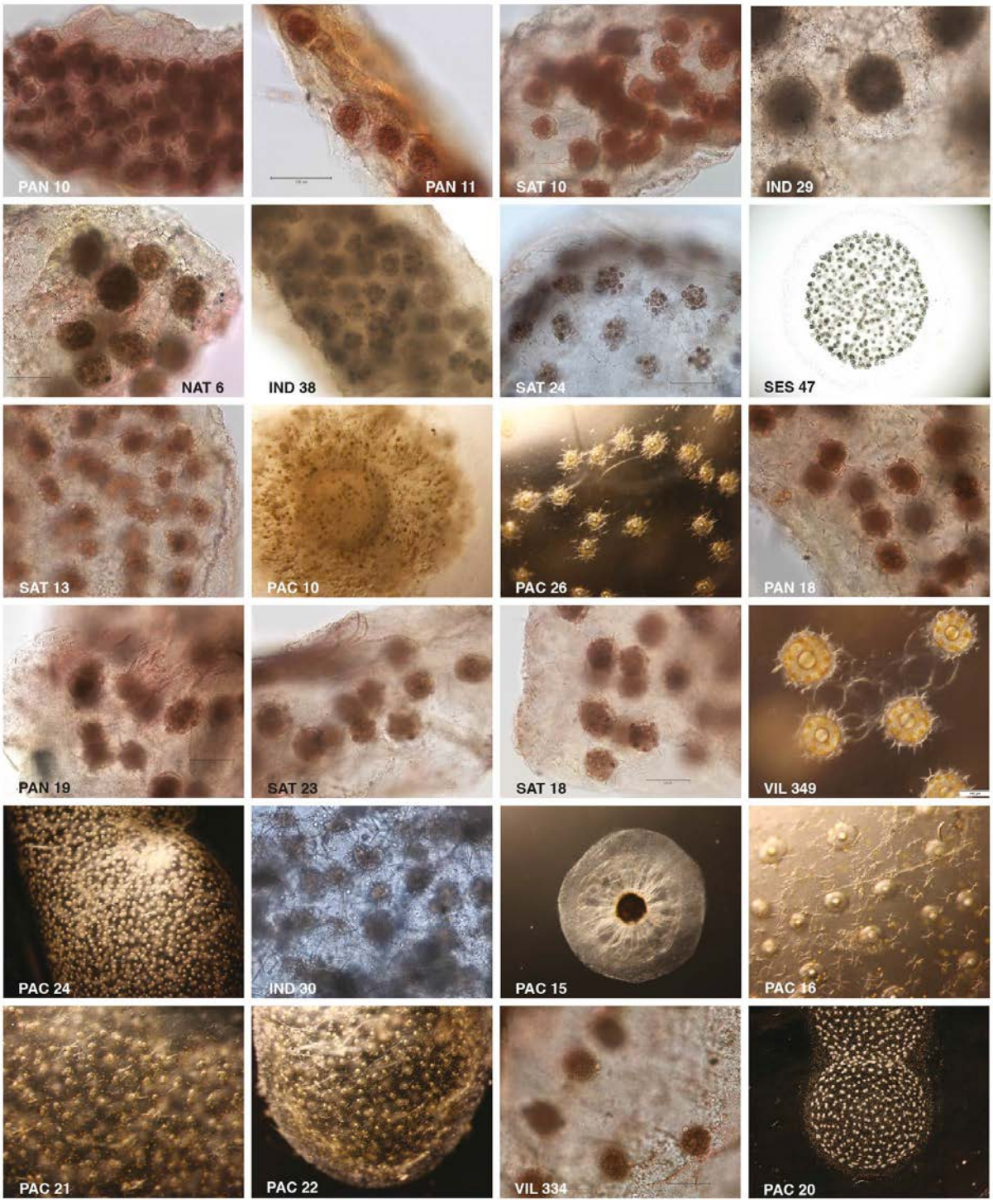
Supplementary Data

Figure S1. Light micrographs of collodarian specimens (species names, clade affiliation and sequence availability are shown in Supplementary Material Table S1).

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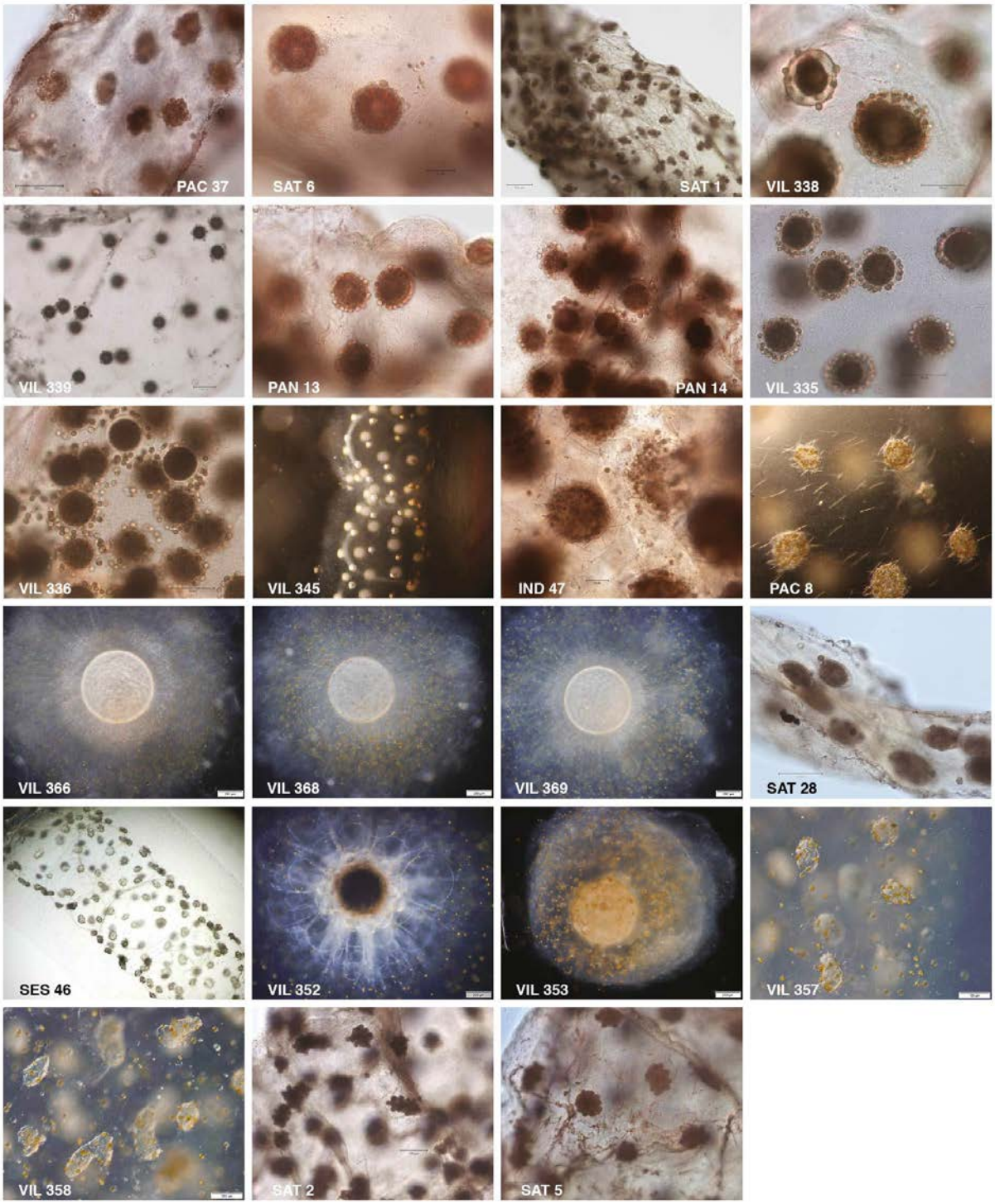
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SAT 5

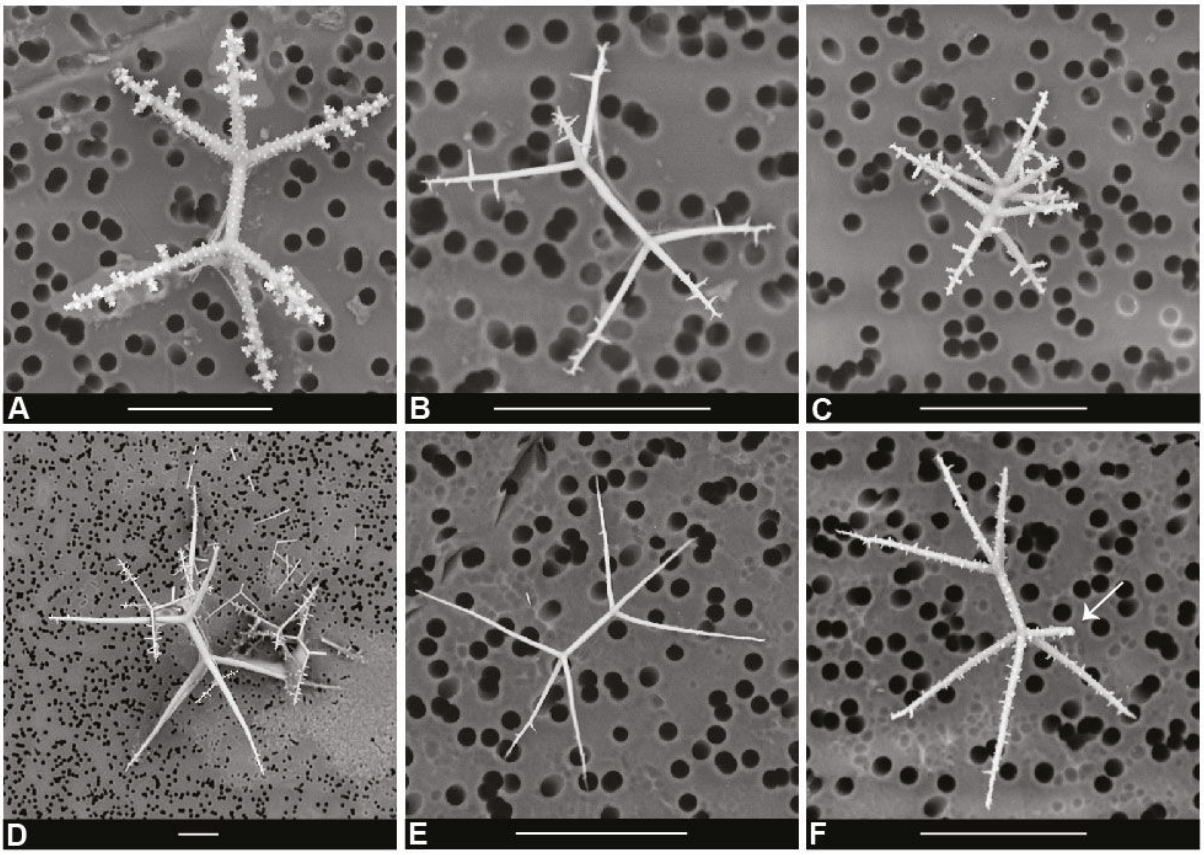

Figure S2. Intraspecific variability of silicified spicules within the Sphaerozoidae. Scanning Electron Microscopy images from the exact same specimens used for the molecular phylogenetic analysis in this study: (**A-D**) *Sphaerozoum armatum* (Pac 24), (**E**) *Sphaerozoum punctatum* (Pac 21) and (**F-G**) *Sphaerozoum trigenimum* (Pan 18). (**A**) Holotype of *S. armatum*. (**B**) Spicule with a smoother surface. (**C**) Abnormal spicule shape with multiple axes. (**D**) Large spicule associates with several smaller spicules inside one specimen of *S. punctatum*. (**E**) Holotype of *S. trigenimum*. (**F**) Appearance of a fourth radiate axis (arrow); scale bars = 30 μm.

Table S1. List of specimens used to obtain collodarian sequences (images of collodarian specimens are shown in Supplementary Material Fig S1).

Specimen ID	Family (shape)	Clade Affiliation	Species (authority)	Sampling Site	18s rDNA Genbank accession number	28s rDNA Genbank accession number
Nat 5	Collosphaeridae (colony)	A1	naked Collosphaeridae	North Atlantic 32°97.791 N, 66°5.466 W	n.a.	KR058258
Pac 12	Collosphaeridae (colony)	A2	naked Collosphaeridae	South Pacific 21°17.462 S, 105°9.476 W	KR058196	KR058259
Pac 17	Collosphaeridae (colony)	A2	naked Collosphaeridae	South Pacific 21°17.462 S, 105°9.476 W	KR058197	KR058260
Pac 39	Collosphaeridae (colony)	A2	naked Collosphaeridae	South Pacific 28°21.665 S, 107°8.749 W	n.a.	KR058261
Sat 12	Collosphaeridae (colony)	A2	*Disolenia quadrata* (Ehrenberg)	South Atlantic 20°31.678 S, 3°2.809 W	KR058198	KR058262
Sat 17	Collosphaeridae (colony)	A3	*Disolenia tenuissima* (Hilmers)	South Atlantic 20°31.678 S, 3°2.809 W	KR058199	n.a.
Sat 19	Collosphaeridae (colony)	A3	*Disolenia tenuissima* (Hilmers)	South Atlantic 20°31.678 S, 3°2.809 W	n.a.	KR058263
Sat 20	Collosphaeridae (colony)	A3	*Disolenia zanguebarica* (Ehrenberg)	South Atlantic 20°31.678 S, 3°2.809 W	KR058200	n.a.
Sat 21	Collosphaeridae (colony)	A3	*Disolenia tenuissima* (Hilmers)	South Atlantic 20°31.678 S, 3°2.809 W	KR058201	KR058264
Sat 26	Collosphaeridae (colony)	A3	*Disolenia zanguebarica* (Ehrenberg)	South Atlantic 8°75.7 S, 17°9.245 W	KR058202	KR058265
Sat 27	Collosphaeridae (colony)	A3	*Disolenia zanguebarica* (Ehrenberg)	South Atlantic 8°75.7 S, 17°9.245 W	KR058203	n.a.
Pac 27	Collosphaeridae (colony)	A5	naked Collosphaeridae	South Pacific 21°17.462 S, 105°9.476 W	KR058204	KR058266
Pac 3	Collosphaeridae (solitary)	A5	*Thalassicolla melacapsa* Haeckel	South Pacific 21°17.462 S, 105°9.476 W	KR058205	n.a.
Pac 7	Collosphaeridae (colony)	A5	*Siphonosphaera abyssi* (Ehrenberg)	South Pacific 21°17.462 S, 105°9.476 W	KR058206	KR058267
Ind 44	Collosphaeridae (colony)	A6	*Collosphaera tuberosa* Haeckel	Indian Ocean 12°80.71 S, 42°2.866 E	KR058207	n.a.
Ind 48	Collosphaeridae (colony)	A6	*Collosphaera tuberosa* Haeckel	Indian Ocean 12°80.71 S, 42°2.866 E	KR058208	n.a.
Pac 2	Collosphaeridae (colony)	A6	*Collosphaera tuberosa* Haeckel	South Pacific 21°17.462 S, 105°9.476 W	KR058209	n.a.
Vil 346	Collosphaeridae (colony)	A6	*Collosphaera tuberosa* Haeckel	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058210	n.a.
Sat 15	Collophidiidae (colony)	B	*Collophidium serpentinium* (Haeckel)	South Atlantic 20°31.678 S, 3°2.809 W	KR058211	KR058268
Sat 25	Collophidiidae (colony)	B	*Collophidium* aff. *ovatum* (Haeckel)	South Atlantic 8°75.7 S, 17°9.245 W	KR058212	KR058269
Ind 46	Collophidiidae (colony)	B	*Collophidium serpentinium* (Haeckel)	Indian Ocean 12°80.71 S, 42°2.866 E	KR058213	KR058270
Sat 4	Collophidiidae (colony)	B1	*Colliphidium ovatum* (Haeckel)	South Atlantic 31°02.92 S, 4°6.742 W	KR058214	KR058271
Pac 1	Collophidiidae (solitary)	B1	*Procyttarium prototypus* Haeckel	South Pacific 21°17.462 S, 105°9.476 W	KR058215	n.a.
Pac 9	Collophidiidae (colony)	B1	*Collophidium* sp.	South Pacific 21°17.462 S, 105°9.476 W	KR058216	KR058272
Ind 20	Collophidiidae (colony)	B2	*Collophidium* cf. *serpentinium* (Haeckel)	Indian Ocean 12°80.71 S, 42°2.866 E	KR058217	KR058273
Pan 10	Collophidiidae (colony)	B2	*Collophidium serpentinium* (Haeckel)	North Pacific 31°52.37 N, 159°0.351 W	KR058218	KR058274
Pan 11	Collophidiidae (colony)	B2	*Collophidium serpentinium* (Haeckel)	North Pacific 31°52.37 N, 159°0.351 W	KR058219	KR058275
Pan 8	Collophidiidae (colony)	B2	*Collophidium* cf. *serpentinium* (Haeckel)	North Pacific 31°52.37 N, 159°0.351 W	KR058220	KR058276
Sat 10	Collophidiidae (colony)	B2	*Collophidium* cf. *serpentinium* (Haeckel)	South Atlantic 20°31.678 S, 3°2.809 W	KR058221	KR058277
Ind 29	Sphaerozoidae (colony)	C1	*Sphaerozoum strigulosum* Breckner in Popofsky	Indian Ocean 12°80.71 S, 42°2.866 E	KR058222	KR058278
Nat 6	Sphaerozoidae (colony)	C1	*Sphaerozoum strigulosum* Breckner in Popofsky	North Atlantic 32°97.791 N, 66°5.466 W	KR058224	KR058280
Ind 38	Sphaerozoidae (colony)	C2	*Sphaerozoum fuscum* Meyen	Indian Ocean 12°80.71 S, 42°2.866 E	KR058223	KR058279
Sat 24	Rozoidae (colony)	C2	*Sphaerozoum fuscum* Meyen	South Atlantic 8°75.7 S, 17°9.245 W	KR058228	KR058285

Ses 47	Sphaerozoidae (colony)	C2	*Sphaerozoum fuscum* Meyen	Sesoko (Japan) 26°37.20 N, 127°5.215 E	KR058229	KR058286
Pac 10	Sphaerozoidae (solitary)	C3	*Thallassicolla melacapsa* Haeckel	South Pacific 21°17.462 S, 105°9.476 W	n.a.	KR058281
Pac 26	Sphaerozoidae (colony)	C3	*Sphaerozoum brandtii* Breckner in Popofsky	South Pacific 21°17.462 S, 105°9.476 W	KR058225	KR058282
Pan 3	Sphaerozoidae (colony)	C3	*Sphaerozoum brandtii* Breckner in Popofsky	North Pacific 11°26.29 N, 152°4.072 W	KR058226	KR058283
Sat 13	Sphaerozoidae (colony)	C3	*Sphaerozoum* sp.	South Atlantic 20°31.678 S, 3°2.809 W	KR058227	KR058284
Pan 18	Sphaerozoidae (colony)	C4	*Sphaerozoum trigenimum* Haeckel	North Pacific 32°99.16 N, 121°8.645 W	KR058230	KR058287
Pan 19	Sphaerozoidae (colony)	C4	*Sphaerozoum trigenimum* Haeckel	North Pacific 32°99.16 N, 121°8.645 W	KR058231	KR058288
Sat 18	Sphaerozoidae (colony)	C4	*Sphaerozoum trigenimum* Haeckel	South Atlantic 20°31.678 S, 3°2.809 W	KR058232	KR058289
Sat 23	Sphaerozoidae (colony)	C4	*Sphaerozoum trigenimum* Haeckel	South Atlantic 8°75.7 S, 17°9.245 W	KR058233	KR058290
Vil 349	Sphaerozoidae (colony)	C4	*Sphaerozoum trigenimum* Haeckel	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058234	n.a.
Pac 24	Sphaerozoidae (colony)	C5	*Sphaerozoum armatum* Haeckel	South Pacific 21°17.462 S, 105°9.476 W	KR058235	KR058291
Ind 30	Sphaerozoidae (colony)	C6	*Sphaerozoum punctatum* (Huxley)	Indian Ocean 12°80.71 S, 42°2.866 E	KR058236	KR058292
Pac 15	Sphaerozoidae (solitary)	C6	*Thalassicolla caerulea* Schneider	South Pacific 21°17.462 S, 105°9.476 W	KR058237	KR058293
Pac 16	Sphaerozoidae (colony)	C6	*Sphaerozoum punctatum* (Huxley)	South Pacific 21°17.462 S, 105°9.476 W	KR058238	KR058294
Pac 21	Sphaerozoidae (colony)	C6	*Sphaerozoum punctatum* (Huxley)	South Pacific 21°17.462 S, 105°9.476 W	KR058239	KR058295
Pac 22	Sphaerozoidae (colony)	C6	*Sphaerozoum punctatum* (Huxley)	South Pacific 21°17.462 S, 105°9.476 W	KR058240	KR058296
Pac 20	Sphaerozoidae (colony)	C7	*Collozoum pelagicum* (Haeckel)	South Pacific 21°17.462 S, 105°9.476 W	KR058241	KR058297
Pac 37	Sphaerozoidae (colony)	C7	*Collozoum pelagicum* (Haeckel)	South Pacific 32°77.523 S, 87°0.971 W	n.a.	KR058298
Pan 17	Sphaerozoidae (colony)	C7	*Collozoum pelagicum* (Haeckel)	North Pacific 32°99.16 N, 121°8.645 W	KR058242	KR058299
Sat 6	Sphaerozoidae (colony)	C7	*Collozoum pelagicum* (Haeckel)	South Atlantic 31°02.92 S, 4°6.742 W	KR058243	KR058300
Vil 334	Sphaerozoidae (colony)	C7	*Collozoum* sp.	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058244	KR058301
Pan 13	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	North Pacific 31°52.37 N, 159°0.351 W	KR058245	KR058302
Pan 14	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	North Pacific 32°68.151 N, 121°9.901 W	KR058246	KR058303
Sat 1	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	South Atlantic 31°02.92 S, 4°6.742 E	n.a.	KR058304
Vil 335	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058247	KR058305
Vil 336	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058248	KR058306
Vil 338	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058249	KR058307
Vil 339	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	n.a.	KR058308
Vil 345	Sphaerozoidae (colony)	C8	*Collozoum inerme* (Müller)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058250	KR058309
Ind 47	Sphaerozoidae (colony)	C9	*Rhaphidozoum acuferum* (Müller)	Indian Ocean 12°80.71 S, 42°2.866 E	KR058251	KR058310
Pac 8	Sphaerozoidae (colony)	C9	*Rhaphidozoum acuferum* (Müller)	South Pacific 21°17.462 S, 105°9.476 W	KR058252	KR058311
Vil 366	Sphaerozoidae (solitary)	C10	*Procyttarium primordialis* (Hertwig)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058253	n.a.
Vil 368	Sphaerozoidae (solitary)	C10	*Procyttarium primordialis* (Hertwig)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058254	n.a.
Vil 369	Sphaerozoidae (solitary)	C10	*Procyttarium primordialis* (Hertwig)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	KR058255	n.a.
Sat 2	Sphaerozoidae (colony)	C11	*Collozoum pelagicum* (Haeckel)	South Atlantic 31°02.92 S, 4°6.742 W	KR058256	KR058312
Sat 28	Sphaerozoidae (colony)	C11	*Collozoum* sp.	South Atlantic 8°75.7 S, 17°9.245 W	n.a.	KR058313
Sat 5	Sphaerozoidae (colony)	C11	*Collozoum pelagicum* (Haeckel)	South Atlantic 31°02.92 S, 4°6.742 W	KR058257	KR058314

Ses 46	Sphaerozoidae (colony)	C11	*Collozoum pelagicum* (Haeckel)	Sesoko (Japan) 26°37.20 N, 127°5.215 E	n.a.	KR058315
Vil 352	Sphaerozoidae (solitary)	C11	*Thalassicolla caerulea* Schneider	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	n.a.	KR058316
Vil 353	Sphaerozoidae (solitary)	C11	*Thalassosphaera spiculosa* Brandt	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	n.a.	KR058317
Vil 357	Sphaerozoidae (colony)	C11	*Collozoum* cf. *longiforme* (Swanberg and Harbison)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	n.a.	KR058318
Vil 358	Sphaerozoidae (colony)	C11	*Collozoum* cf. *longiforme* (Swanberg and Harbison)	Villefranche-sur-Mer (France) 43°41.10 N, 7°1.850 E	n.a.	KR058319

Table S2. List of environmental sequences related to Collodaria.

Accession number	Associated family	Associated sub-clade	Reference	Location
GU825502	Collosphaeridae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823877	Collosphaeridae	-	Edgcomb et al. (2011)	Cariaco Basin
FN598273	Collosphaeridae	A1	Sauvadet et al. (2010)	South Pacific
GU824776	Collosphaeridae	A5	Edgcomb et al. (2011)	Cariaco Basin
GU824098	Collosphaeridae	A5	Edgcomb et al. (2011)	Cariaco Basin
AY256280	Collosphaeridae	A5	Stoeck et al. (2003)	Cariaco Basin
AF530524	Collophidiidae	-	Lopez-Garcia et al. (2003)	Hydrothermal Mid Atlantic Ridge
AY256264	Collophidiidae	-	Stoeck et al. (2003)	Cariaco Basin
AY882496	Collophidiidae	-	Stoeck et al. (2006)	Cariaco Basin
FN598237	Collophidiidae	-	Sauvadet et al. (2010)	South Pacific
FN598300	Collophidiidae	-	Sauvadet et al. (2010)	South Pacific
GU820939	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU821759	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU822336	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823698	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823748	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823751	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823782	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823846	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823866	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823935	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823938	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU823950	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824024	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824030	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824065	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824080	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824113	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824209	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824240	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824243	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824256	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824258	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824264	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824278	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824360	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824375	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824379	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824441	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824450	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824484	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824487	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824488	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824497	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824572	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824619	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824625	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824626	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824702	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824724	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824747	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824759	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824761	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824762	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824777	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824925	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU824964	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin

GU825011	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825013	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825032	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825058	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825096	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825134	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825203	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825233	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825241	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825257	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825285	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825287	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825331	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825342	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825348	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825354	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825397	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825418	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825420	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825452	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825489	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825496	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825512	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825516	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825590	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825611	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825621	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825711	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
GU825728	Collophidiidae	-	Edgcomb et al. (2011)	Cariaco Basin
JX194712	Collophidiidae	-	Jungbluth et al. (2013)	Juan de Fuca Ridge flank
AY046714	Collophidiidae	B1	Edgcomb et al. (2002)	Guaymas Basin
GU825193	Collophidiidae	B2	Edgcomb et al. (2011)	Cariaco Basin
GU825419	Collophidiidae	B2	Edgcomb et al. (2011)	Cariaco Basin
IBEA.CTG.2022727	Sphaerozoidae	C8	Venter et al. (2004)	Sargasso Sea
EF172833	-	-	Not et al. (2007)	Sargasso Sea

CHAPITRE II

BIODIVERSITY OF THE COLLODARIA

« But, beneath the waves, there are many dominions yet to be visited, and kingdoms to be discovered; and he who venturously brings up from the abyss enough of their inhabitants to display the physiognomy of the country, will taste that cup of delight, the sweetness of whose draught those only who have made a discovery know. »

Edward Forbes (1859)

CHAPITRE II-1

Global biogeography of Collodaria (Radiolaria) assessed through metabarcoding

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Abstract

Collodaria are heterotrophic marine protist which exist either as large colonial specimens made of hundreds cells or as large solitary cells, and all described species so far harbour intracellular photosynthetic microalgae as photosymbionts. Although recent environmental molecular diversity surveys demonstrated their important contribution to planktonic communities and worldwide occurrence, our understanding of their diversity and ecology is still very limited. Here we estimated the 18S rDNA copies per cell of solitary and colonial collodarians using single-cell quantitative qPCR and found high values in colonies (37 474 ±17 799 SSU rDNA copies) while solitary collodarian display lower number (5 770 ±1 960). We then investigated the environmental diversity of Collodaria by using a V9-18S rDNA metabarcoding survey from the *Tara* Oceans Expedition and found that the two collodarian families Collosphaeridae and Sphaerozoidae, contributed the most to the collodarian diversity and encompassed mostly cosmopolitan taxa. Although the biogeographical patterns were homogeneous, we observed that coastal biogeochemical biomes were consistently less diverse than oceanic biomes and were dominated by the Sphaerozoidae while the Collosphaeridae were dominant in the open-oceans. The significant correlations observed with 6 environmental variables finally suggested that collodarian diversity increased towards the more oligotrophic regions.

Introduction

Radiolarians are skeleton-bearing marine heterotrophic protists belonging to the eukaryotic phylum Retaria within the super-group Rhizaria (Nikolaev *et al.*, 2004; Adl *et al.*, 2005; Moreira *et al.*, 2007). The Radiolaria encompass more than 700 extant species, classified in five well-established orders, among which the Acantharia possess a skeleton made of strontium sulfate, the Taxopodia, Collodaria, Nassellaria and Spumellaria have a skeleton made of opaline silica (Suzuki and Not, 2015). As they are particularly difficult to maintain alive in culture, most of our knowledge on radiolarians come from paleontological studies (Wever *et al.*, 2002) and less is know about their actual diversity and ecology in modern oceans. Among radiolarian, Collodaria is a particularly poorly studied group in both paleontological and biological oceanography studies.

Collodarians can be observed either as colonial or solitary forms. The size of a colony ranges from a few millimetres to a maximum recorded of three meters (Swanberg and Harbison, 1979). Each colony is composed of hundreds to thousands of collodarian cells embedded in a gelatinous matrix while the solitary collodarians are composed of a single cell. Although very little is know with respect to the feeding behaviour of Collodaria, all species reported so far harbour numerous symbiotic microalgae (photosymbionts), mostly identified as the dinoflagellate *Brandtodinium nutricula* (Hollande and Enjumet, 1953; Probert *et al.*, 2014). Taxonomical delineation of the different collodarian species is challenging due to their limited number of morphological criterion (Brandt, 1885; Haeckel, 1887). Recently, an integrative taxonomy approach allowed to better understand the diversity of Collodaria, clearly distinguishing three monophyletic families (Collosphaeridae, Collophidiidae and Sphaerozoidae) and twenty clades including both solitary and colonial species (Biard *et al.*, 2015).

So far, only a few studies have described the geographical distribution of collodarian taxa throughout the world ocean and reported that Collodaria are globally distributed across a large variety of marine environments (Pavshtiks and Pan'kova, 1966; Strelkov and Reshetnyak, 1971; Swanberg, 1979). They preferentially inhabit the near surface of oligotrophic waters where they can be locally abundant, from 30 to exceptionally 20 000 colonies m^{-3} (Khmeleva, 1967; Caron and Swanberg, 1990; Dennett *et al.*, 2002). Recent *in situ* estimation of collodarian abundances highlighted that Collodaria were the main contributors to the rhizarian biomass in the upper 100 meters of the oceans (Biard *et al.*, submitted). Despite their contribution to zooplankton biomass, the contribution of photosymbiotic collodarians to total primary production is rather low (i.e. locally up to 1%; Caron *et al.*, 1995), but also unique with regards to the trophic position of Collodaria (Swanberg, 1979). As recent studies unravelled the importance of Collodaria in marine ecosystems (Lima-Mendez *et al.*, 2015; Villar *et al.*, 2015; Biard *et al.*, submitted; Guidi *et al.*, submitted), our understanding of the collodarian biodiversity, its extent and distribution, is paradoxically still very limited.

In the last decades, environmental molecular diversity surveys based on the 18S rRNA gene, regularly highlighted a high diversity and a relative important contribution of radiolarians to planktonic communities in marine ecosystems (Countway *et al.*, 2007; Not *et al.*, 2007; Sauvadet *et al.*, 2010; Edgcomb *et al.*, 2011) and in particular of the Collodaria, from photic layers (de Vargas *et al.*, 2015) to the bathypelagic regions of the oceans (Pernice

et al., 2015). Similar molecular approaches applied to the analyses of protist communities collected by sediment traps also highlighted the important contribution of Collodaria in the particle export to the deep ocean (Amacher *et al.*, 2009; Fontanez *et al.*, 2015; Guidi *et al.*, submitted). Yet these studies generally lacked taxonomic resolution, as no reliable reference database for the 18S rDNA was available for detailed assignation of Collodaria, but also did not consider the quantification of the collodarian rDNA copy number in each cells, a parameter largely variable among marine protists (Zhu *et al.*, 2005; Godhe *et al.*, 2008) and which can have a non negligible impact at the time of analysing large amount of high-throughput sequencing data from the environment.

In this study we investigated the global biogeography of the Collodaria across a variety of marine ecosystems sampled during the *Tara* Oceans expedition (Pesant *et al.*, 2015). Our analyses were based on a newly defined reference framework (Biard *et al.*, 2015) and considering the number of rDNA copies per central capsule in different collodarian species, including colonial and solitary organisms. We also investigated the relationships between the distribution of collodarian diversity across a variety of biogeochemical biomes and a set of 15 environmental variables.

Material and methods

Real-time quantitative PCR analysis of single-cell collodarian

Colonial and solitary collodarian specimens were collected in the bay of Villefranche-sur-Mer (France) using a Regent Net (680 µm mesh size) or a hand net (Supplementary Figure S1). Each specimen was micropipette isolated, cleaned into 0.2 µm filtered seawater and imaged under a binocular microscope. DNA from each specimen was extracted, amplified and the 18S rRNA gene was sequenced using the set of primers S32col/V9R as previously described (Biard *et al.* 2015). Two colonial species, *Sphaerozoum fuscum* and *Collozoum pelagicum*, and one solitary species *Procyttarium primordialis*, were identified based on morphological and molecular identity according to the latest classification of Collodaria (Biard *et al.*, 2015).

In order to avoid eukaryote contaminations from preys or microalgal photosymbionts, we designed two collodarian specific-primers, Col-961-1F (5'-CAR CTA GGG GTT GGC AAA T-3') and Col-1075R (5'-CAC ATC TTG TGG TGC CCT T-3'). Primers were designed and optimized using a reference alignment of 38 Sphaerozoidae 18S rDNA sequences, and using PrimaClade (Gadberry *et al.*, 2005) and the OligoAnalyzer 3.1 software program (Integrated DNA Technologies). The specificity of the newly designed primers was evaluated by PCR using genomic DNA from Acantharia, Nassellaria, Spumellaria, and the collodarian photosymbiont *Brandtodinium nutricula*. PCR was performed as previously described (Biard *et al.*, 2015).

We used the Col-961-1F/Col-1075R primer set to PCR amplify a 114-bp fragment of a *Collozoum inerme* (accession no. KR058247) to be used as standard for qPCR assays. The amplicon was cloned into *E. coli*. Plasmid DNA was extracted using the NucleoSpin Plasmid (NoLid) kit (Macherey-Nagel, Hœrdt, France) and newly constructed plasmids were linearized using NotI enzyme. Linearized plasmids were analysed by electrophoresis in 1% agarose gel and concentration measured using a Qubit Fluorometer (Fischer Scientific,

Illkirch, France). The number of copies in the standard was calculated as previously described (Zhu *et al.*, 2005). A serial tenfold dilutions (10^{-1} to 10^{-6}) were used to obtain standard curves. All reactions were performed in technical duplicate with a LightCycler 480 Real-Time PCR System (Roche, Boulogne-Billancourt, France), using the LightCycler 480 SYBR Green I Master kit (Roche). Reactions were performed by denaturing at 95°C for 4 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C and extension at 72°C for 10 s, respectively. Data were retrieved at the extension step.

Metabarcoding sample acquisition and processing

The environmental diversity of Collodaria was explored in 653 samples (including 4 size fractions, 0.8-5 µm, 5-20 µm, 20-180 µm and 180-2 000 µm) collected at the surface and the depth of the chlorophyll maximum in 113 stations (Supplementary Figure S2). Samples were collected with plankton nets, pump or Niskin bottles, formerly described (Pesant *et al.*, 2015). The V9-18S rDNA metabarcodes were extracted for each samples and processed with a bioinformatics pipeline previously described (de Vargas *et al.*, 2015). Briefly, the pipeline consisted in 1) quality checking, 2) filtering (metabarcodes present in less than 2 samples and with less than 3 reads were removed) and 3) clustering into operational taxonomic units (OTUs) using the 'swarm' method (Mahé *et al.*, 2014). From this, the OTUs were assigned by comparison to the Protist Ribosomal Reference (PR2) database (Guillou *et al.*, 2013) modified with the inclusion of new collodarian reference sequences (Biard *et al.*, 2015). For OTUs having contentious assignation (e.g. several matches with different reference sequences, or low hit-score), they were classified as uncertain. We finally eliminated the sampling stations having less than 2 samples to allow a better reproducibility of the results.

To determine the most relevant identity threshold to analyse the collodarian biodiversity, we calculated pairwise identity values for the full-length 18S rRNA gene and its hypervariable regions V4 and V9 (Supplementary Figure S3), using the *'seqidentity'* function implemented in the 'bio3d' package (Grant *et al.*, 2006). The reference alignment used for this analysis comprised 81 collodarian 18S rDNA sequences, representing the most exhaustive collodarian dataset up to date. We consequently extracted only the collodarian OTUs with ≥80 % identity to a reference sequence.

Data analyses

All data analyses and statistics described below were performed using R 3.2.0. (R Core Team, 2015) and the ggplot2 (Wickham, 2009), Hmisc 3.16-0 (Harrell, 2015), and vegan 2.3-0 (Oksanen *et al.*, 2015) packages as well as custom scripts. For each sampling stations, we did not found significant differences in OTU composition between the 4 different size fractions nor the 2 depths, and consequently pooled all the sequences from different samples collected in the same sampling station for statistical analyses of the OTU composition and richness. To investigate the similarity in OTU composition between sampling stations, we performed a nonmetric multidimensional scaling (NMDS) of Jaccard distances between the sampling stations, by transforming the data to presence-absence prior to the analyses.

We created an environmental dataset composed of 15 environmental variables available online at PANGEA (http://doi.pangaea.de/10.1594/PANGAEA.840718), recorded at the surface and the DCM, to investigate their relationships with the collodarian biodiversity. For

each sampling stations, we calculated the average value of each environmental variable. Collodarian diversity (expressed here as the OTU richness), as well as other environmental variables, were log-transformed to normalize them and we calculated the Pearson correlation coefficient between pairs of variables. Because of the increased risk of a type I error when several tests of significance are performed simultaneously (Legendre and Legendre, 2012), we used the sequential Bonferroni adjustment to test the significance of the correlation coefficients (Rice, 1989).

Results

Quantification of 18S rRNA gene copy number in colonial and solitary Collodaria

We quantified the number of 18S rRNA gene copies of two colonial and one solitary collodarian species (Table 1). Overall, when reported to the number of cells (i.e. estimated by the number of central capsules forming the colony), colonies showed about 7 folds more copies than solitary specimens, 37 474 ±17 799 (mean ±SEM) and 5 770 ±1 960, respectively. The rDNA copy numbers between the two different colonial species appeared lower in *S. punctatum* (26 189 ±4 849) than in *C. pelagicum* (45 534 ±6 767). We then compared the estimates for collodarian 18S rDNA copy numbers with previous estimates of copy numbers for a broad range of marine protist, extracted from the literature (Supplementary Table S1). The number of rDNA copies was significantly correlated with cell length (F = 251, R^2_{adj} = 0.76, *p-value* < .001; Figure 1). While all colonial specimens match to the general pattern, the solitary specimens (*Procyttarium primordialis*) display relatively low rDNA copy numbers compared to their large cell size.

Table 1 Quantification of rDNA copy numbers in colonial and solitary Collodaria with quantitative PCR.

Organism (ID n°)	Clade affiliation	Accession No. (SSU)	Organism length (mm)	Central capsules (n)*	Central capsule size (µm)**	Copies per central capsule (n)	Standard Deviation
Colonial collodarians							
Sphaerozoum fuscum (1)	C2	XXXXXXX	8.25	225	118 ±23	14 933	667
Sphaerozoum fuscum (2)	C2	XXXXXXX	5.77	200	112 ±13	23 275	575
Sphaerozoum fuscum (3)	C2	XXXXXXX	3.67	114	96 ±14	32 895	1 316
Sphaerozoum fuscum (4)	C2	XXXXXXX	4.09	134	104 ±10	18 470	37
Sphaerozoum fuscum (5)	C2	XXXXXXX	4.49	131	112 ±9	41 374	3 740
Mean	-	-	-	-	-	**26 189**	**4 849**
Collozoum pelagicum (1)	C8	XXXXXXX	12.17	173	89 ±10	32 977	2 572
Collozoum pelagicum (2)	C8	XXXXXXX	8.10	151	87 ±12	74 834	0
Collozoum pelagicum (3)	C8	XXXXXXX	3.44	100	68 ±9	47 950	1 150
Collozoum pelagicum (4)	C8	XXXXXXX	10.29	245	77 ±8	28 755	633
Collozoum pelagicum (5)	C8	XXXXXXX	7.28	111	85 ±11	60 450	991
Collozoum pelagicum (6)	C8	XXXXXXX	8.20	196	86 ±10	25 765	153
Collozoum pelagicum (7)	C8	XXXXXXX	4.82	108	73 ±13	48 009	1 806
Mean	-	-	-	-	-	**45 534**	**6 767**
Mean for all colonial collodarians	-	-	-	-	-	**37 474**	**17 799**
Solitary collodarians							
Procyttarium primordialis (1)	C10	XXXXXXX	4.96	1	661	1 790	90
Procyttarium primordialis (2)	C10	XXXXXXX	2.95	1	374	5 355	275
Procyttarium primordialis (3)	C10	XXXXXXX	3.94	1	370	13 250	450
Procyttarium primordialis (4)	C10	XXXXXXX	2.64	1	370	3 980	80
Procyttarium primordialis (5)	C10	XXXXXXX	4.69	1	570	4 475	75
Mean	-	-	-	-	-	**5 770**	**1 960**

* Central capsules are proxies for the number of collodarian cells.

** For colonial collodarian, the central capsule size is the average size (±standard deviation) estimated from the measurement of 15 randomly selected central capsules.

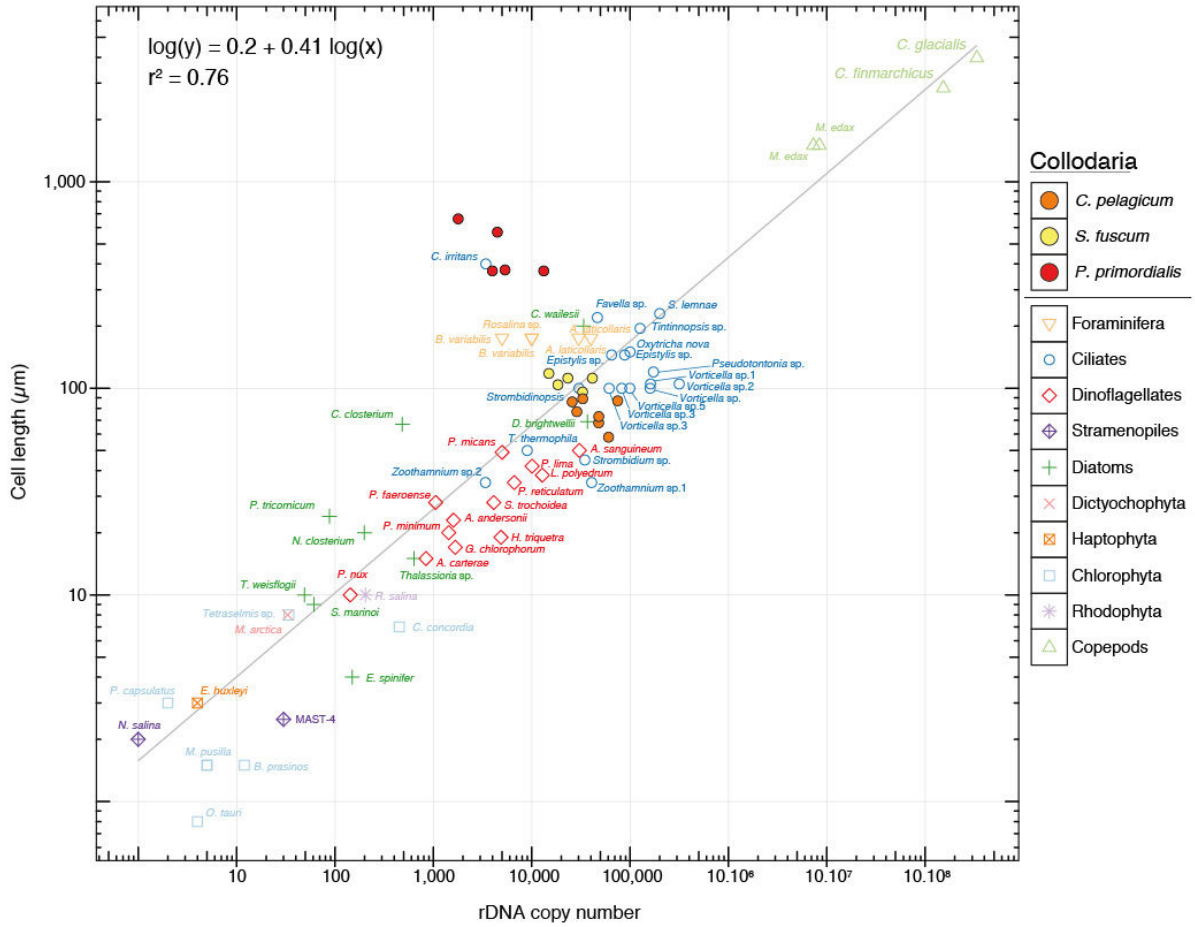

Figure 1 Correlation between the rDNA copy number per cell estimated by quantitative PCR and the cell length across eukaryotic taxa including the three different collodarian species (filled circles). Detailed measurements are provided in Supplementary Table S1.

Biogeography of Collodaria in the global ocean

At a first cut-off threshold of 80% identity to the sequences included in the PR2 database (Guillou *et al.*, 2013), a total of 193 610 819 V9 rDNA sequences were assigned to the Rhizaria and further grouped in 22 192 operational taxonomic units (OTUs) using the *swarm* method (Mahé *et al.*, 2014). Out of these sequences, and using a relative abundance threshold at 1 000 sequences based on our qPCR analysis, Collodaria accounted for a total of 133 301 730 sequences and 230 OTUs. The use of this second threshold led to the removal of 68 samples and 7 sampling stations, where we no longer retrieved rhizarian OTUs, having relative sequence abundances lower than 1 000 sequences. When considering Collodaria, we removed another 12 sampling stations, into which no collodarian sequences were found, from our analysis (Supplementary Figure S2). Based on relative sequence abundances, we estimated that Collodaria accounted for an average of 82% of the total rhizarian sequences and 64% of the rhizarians OTUs (Figure 2). Collodaria were the dominant lineage within the Rhizaria within all biomes, except in the Antarctic biome, where 3 sampling stations completely lacked Collodaria. Overall, variations of collodarian contribution to the rhizarian sequences matched very well their contribution to rhizarian OTUs, except for a few sampling stations (e.g. station 43 in the Indian Ocean) where the collodarian contribution to rhizarian OTUs was lower than their contribution to rhizarian sequences.

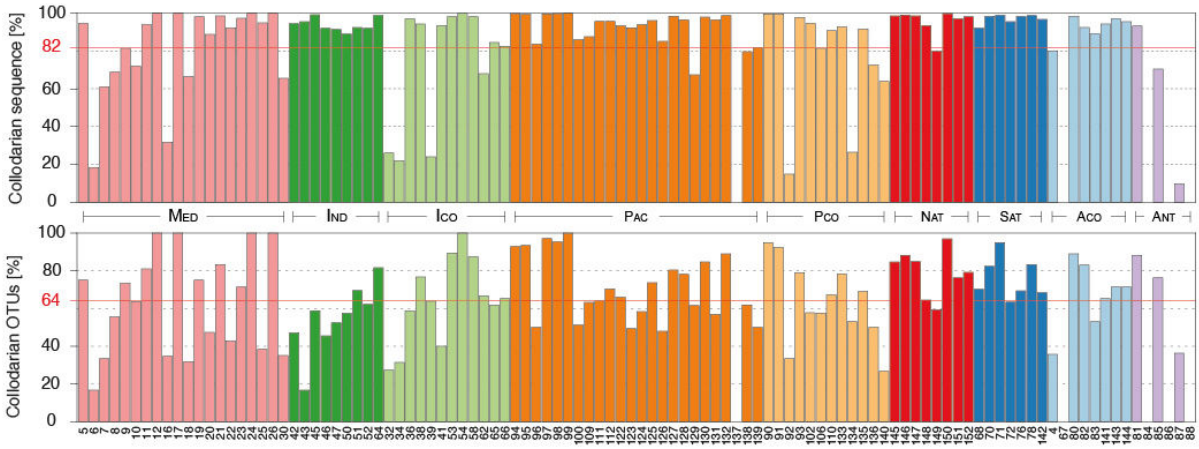

Figure 2 Contribution of Collodaria to the Rhizaria lineage across the *Tara* Ocean sampling stations. *Upper panel:* Contribution of Collodaria to the total number of Rhizaria sequences. *Lower panel:* Contribution of Collodaria to the total number of Rhizaria OTUs. The red lines display the mean contributions for each dataset. Contributions are geographically divided according to Longhurst's Biogeochemical Biomes (Longhurst, 2010).

The latitudinal variations of collodarian OTU richness revealed a hump-shaped relationship centred between 10-30° S and exhibiting higher diversity at low latitudes (Figure 3a). Additionally, the collodarian richness greatly varied within and between the different biogeochemical biomes, reaching the highest average value (40 OTUs) in the Atlantic Trade Wind Biome (Sat; Figure 3b). The Mediterranean Sea (Med) was the least diverse biome, with an average richness of 12 OTUs. Overall, coastal biomes (i.e. Med-Ico-Pco-Aco) had a significantly lower OTU richness (M = 17.26, SD = 13.28) compare to the open ocean biomes (i.e. Ind-Pac-Nat-Sat-Ant) (M = 30.29, SD = 15.05), $t(93) = 4.48$, $p < .001$. For each of the 3 oceanic basins, Indian Ocean, Pacific Ocean and Atlantic Ocean (North and South), we compared the OTU richness between coastal and oceanic biomes (Figure 3b). Although we observed a small level of statistical difference ($p < .05$ and $p < .01$ respectively) between the coastal and oceanic sampling stations for the Pacific and Atlantic Oceans, we did not observed any significant difference within the Indian Ocean.

The environmental diversity of OTUs assigned to the Collodaria covered all the currently recognized collodarian families. For 89 OTUs having uncertain assignations with the procedure used for the entire dataset, we identified the closest environmental and cultured match using the NCBI BLASTN tool and assigned them to the Collophidiidae (41 OTUs), the Sphaerozoidae (37) and the Collosphaeridae (11) according to the established collodarian reference database (Supplementary Table S2; Biard *et al.*, 2015). When considering all the OTUs together, the Collosphaeridae (Clade A) was the most abundant family, accounting for an average of 63.41% of all collodarian sequences (Figure 4a). Although it was less abundant (31.46% of all sequences), the Sphaerozoidae (Clade C) was the most diverse family encompassing almost half of all collodarian OTUs (109 OTUs), while the Collosphaeridae only gathered 72 OTUs (Figure 4a). The third family, the Collophidiidae (Clade B), showed an overall low abundance (5.13%) and was fairly diverse (49 OTUs; Figure 4a). We further distinguished 13 clades among the three families, clades A4, B1 and C7 being the most

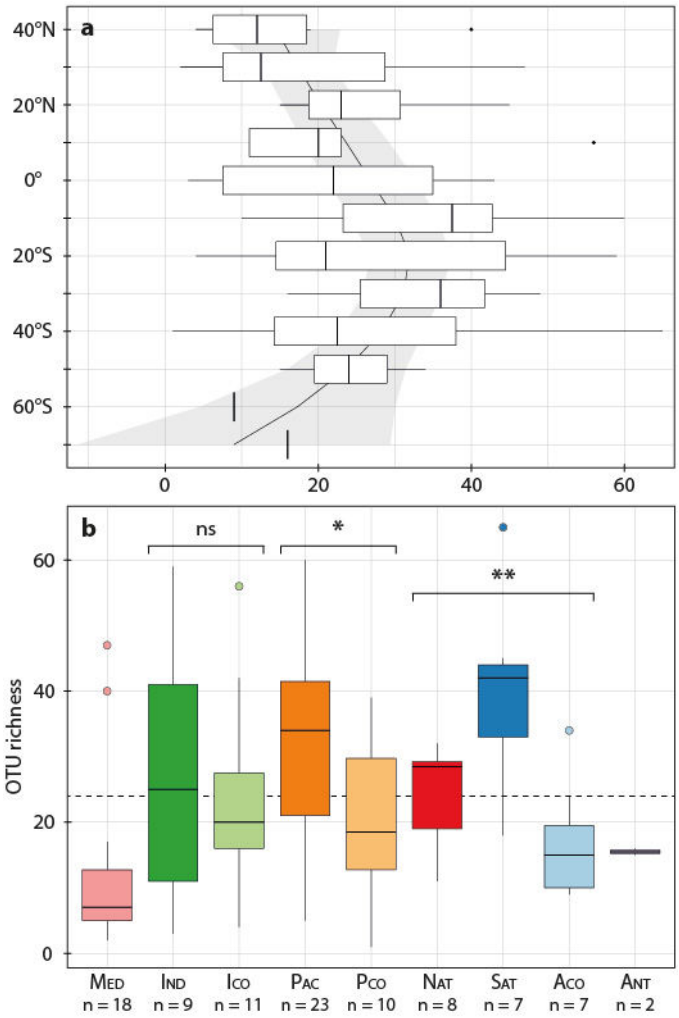

Figure 3 Variation in collodarian diversity across oceanic basins and latitudes. (**a**) Latitudinal distribution of collodarian OTU richness. Loess regression with polynomial fitting was computed to illustrate the latitudinal pattern. Pale colour area displays a 0.95 confidence interval around the trend. (**b**) Variation of OTU richness across Longhurst's biomes. Sample size (n) for each biome is indicated along the x-axis. The dashed line represents the overall mean OTU richness (S = 24). *P-value* (ns: *p-value* > .05, one star: *p-value* < .05, two stars: *p-value* < .01) for Welch-test show the significant decrease in OTU richness between coastal and oceanic biomes.

abundant and, clades A5, B1 and C7 being the most diverse clades within each family (Figure 4a). Overall, the clade A4 was the most abundant with 21.59% of all collodarian sequences.

We observed that the most abundant OTUs were ubiquitous across the sampling stations (Figure 4b) and that each of the 15 most abundant were distributed at least in 32 sampling stations among a total of 95 (i.e. 34%). A few other 'rare' OTUs occurred in a limited number of sampling locations, typically being observed in 2 or 3 sampling stations.

The geographical distribution of the different collodarian families and clades among 9 different biogeochemical regions, revealed a rather homogeneous distribution, with a few clades (1 or 2) prevailing at each sampling station (Figure 5). On average, the Collosphaeridae appeared dominant (65-76%) in open ocean waters (i.e. Ind, Pac, Nat, Sat and Ant), while we observed a rather clear domination (45-66%) of the Sphaerozoidae at coastal biomes (i.e. Ico, Pco and Aco), with the exception of the Mediterranean Sea (Med) clearly dominated by the Collosphaeridae (77%; Figure 5). Collophidiidae were rare, accounting for less than 10% of the collodarian sequences in all biomes, with the exception of a few sampling stations (e.g. 132 and 139, both in the Pacific Trade Wind Biome) where they displayed significantly higher contribution (Figure 5). Within each biome, the distribution patterns were rather homogeneous across sampling stations except for the Mediterranean Sea where a clear shift appeared in clade composition when going from the occidental basin (sampling stations 5-12) to the oriental basin (17-30), the Sphaerozoidae being dominant in the western part of the sea (48% of collodarian sequences) and replaced by the Collosphaeridae in the eastern part (84% of collodarian sequences).

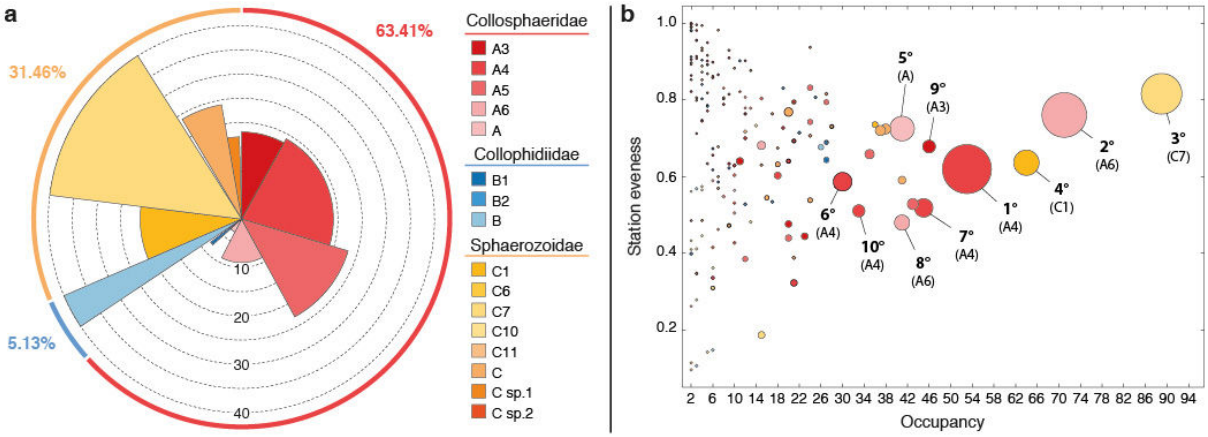

Figure 4 Diversity of the Collodaria (**a**) Contribution of the different clades to the total collodarian OTUs (height = number of OTUs) and the total number of collodarian sequence (radius = % contribution) (**b**) Occupancy and evenness of each collodarian V9 rDNA OTU identified across stations sampled. Low evenness indicates that an OTU is not equally distributed over the sampling stations where it has been observed and vice versa. Each circle represents one of the collodarian V9 rDNA OTU with its size being proportional to the total read abundance. The 10 most abundant OTUs are labelled according to their rank and taxonomic clade affiliation.

We then investigated the variation in collodarian diversity in regards to a set of environmental variables (averaged over the surface and DCM) available for the samples considered (Supplementary Table S4). Whether considering the 16 clades or the 3 families illustrated in the dataset, their relationships with environemental variables were not statistically significant, yet we observed increasing or decreasing trends for a number of diffent variables (Supplementary Figure S4). Instead of taxonomic groups, when considering the log-transformed collodarian OTU richness, we found significant relationships with six environmental variables, the bathymetry (i.e. bottom depth), the distance to the coast, the depth of the mixed-layer, the backscattering coefficent of particles, the silica concentration and the depth of the chlorophyll maximum (Figure 6 and Supplementary Table S4). The correlation coefficient between collodarian diversity and the bathymetry of each sampling station, was the highest, $r(93) = 0.5456$, $p < .001$ (Figure 6a) and explained 29% of the total variation in collodarian diversity. Although the data were rather scattered on the plots, the OTU richness showed significant increases with the following log-transformed variables: the distance to the coast, $r(92) = 0.4706$, $p < .001$ (Figure 6b), the depth of the mixed-layer, $r(90) = 0.4360$, $p < .001$ (Figure 6c), the silica concentration, $r(86) = 0.3223$, $p < .01$ (Figure 6e) and the depth of the deep chlorophyll maximum, $r(79) = 0.2973$, $p < .01$ (Figure 6f). The OTU richness showed a significant decrease with the log-transformed backscattering coefficient of particles, a proxy for the particulate organic carbon, $r(80) = -0.3933$, $p < .001$ (Figure 6d).

Discussion

Quantifying the rDNA copy numbers

Using specifically designed qPCR primers, we determined the number of 18S rRNA gene copies for 17 collodarian specimens, encompassing three different species (Table 1). The 7 times higher 18S rRNA gene copy number observed between the colonial compare to the

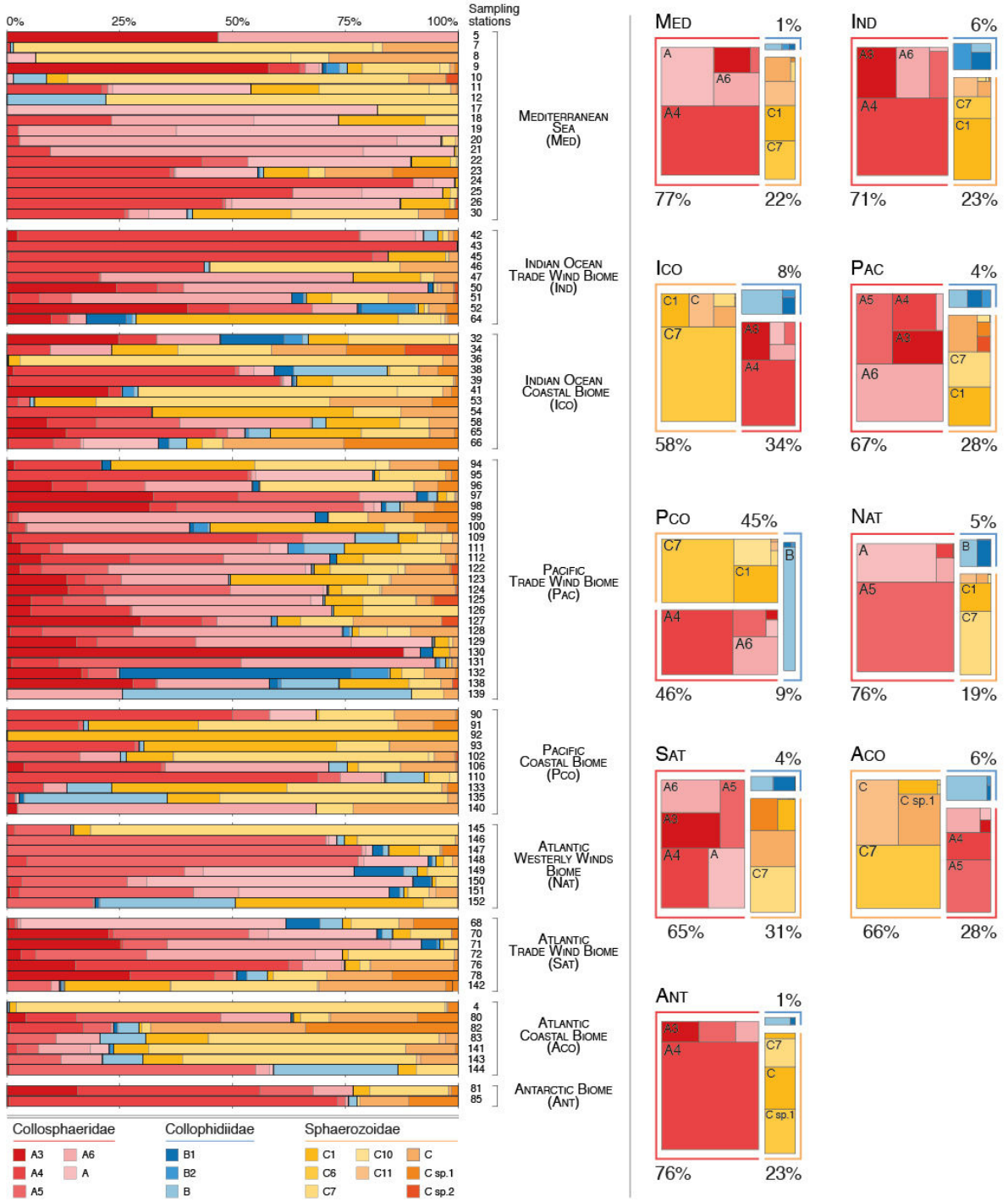

Figure 5 Relative abundance of rDNA metabarcodes assigned to the different collodarian families and clades defined previously (Biard *et al.*, 2015) and across the *Tara* Oceans sampling stations. Relative contributions are geographically divided according to the Longhurst Biomes (Longhurst, 2010). For each biome, average contributions of the different collodarian families are shown on the right panel and the 5 most abundant clades are labelled according to their affiliation. Detailed numerical values are shown in Supplementary Table S3.

solitary specimens could be explained by the presence of multiple nuclei, estimated from 4 to 100 nuclei per cells in colonial forms (Suzuki *et al.*, 2009), while solitary specimens possess a single nucleus (Huth, 1913; Anderson, 1976; Suzuki *et al.*, 2009). Other hypotheses, such as the large size of solitary central capsule leading to a small copy number/size ratio or the

difference in ploidy between the two forms, the solitary being an haploid stage of the colonial specimens (Biard *et al.*, 2015), cannot be ruled out completely.

The number of rDNA copies weighted by the number of central capsules (i.e. actual number of cells) showed a distribution consistent with other marine protists (Figure 1), and similar compared to the Foraminifera (5 000 – 40 000 of 18S rRNA gene copies; Weber and Pawlowski, 2013), their closest relative in the overall comparison in the rDNA copy content in marine protists (Supplementary Table 1). Our results confirm and fit the previously reported correlation between size and number of rDNA copies (Zhu *et al.*, 2005; Godhe *et al.*, 2008). When considered the full specimen, colonial collodarian displayed the highest rDNA copy number ever recorded in any marine protist. Among the 12 colonial specimens we analysed here, the highest number of rDNA copies (11 300 000 rDNA copies) was recorded for a *Collozoum pelagicum* colony measuring 8 x 2 mm (~14 mm^2). Such high content is almost similar to the number of rDNA copies estimated in the multicellular crustacean copepod *Mesocyclops edax* (Supplementary Figure S5; Wyngaard *et al.*, 1995). As colonial collodarian often display sizes larger than several centimetres, up to a few meters (Swanberg and Harbison, 1979), we estimate that a 400 x 2 mm cylindrical colony (i.e. 2 512 mm^2, the size of a colony reported in Swanberg and Harbison, 1979) with a central capsule density of 9.12 capsule mm^{-2} (Dennett *et al.*, 2002) could possess almost 1 billion rDNA copies. These high values illustrate the difficulty to appreciate the real significance of Collodaria in metabarcoding surveys, as they can potentially lead to an overestimation of their importance, depending on the care with which the samples have been collected. Indeed, colonial Collodaria are easily broken upon collection with plankton net or during filtration procedures, and the high rDNA copy content could propagate to smaller size-fractions (e.g. 0.8-5 µm), where the high diversity and contribution of Collodaria have been reported on several occasions (Not *et al.*, 2007; Sauvadet *et al.*, 2010; Edgcomb *et al.*, 2011; Massana, 2011).

Refining the dataset accuracy

Interpretations of data from metabarcoding diversity surveys depend on a number of methodological and analytical processes such as PCR artefacts and sequencing errors, which are known to inflate diversity estimates (Kunin *et al.*, 2010; Lee *et al.*, 2012), quality control procedures of the sequences generated (Huse *et al.*, 2010; Quince *et al.*, 2011), and intra-genomic polymorphism, recently demonstrated in several rhizarian taxa (i.e. Foraminifera, Acantharia), including the Nassellaria, the closest relative to Collodaria in molecular phylogenies (Pillet *et al.*, 2012; Decelle *et al.*, 2014). Using intraspecific variability estimates across collodarian families (Supplementary Figure S3) and an assessment of the 18S rRNA gene copy number in single-cell (Table 1), we defined a total of 230 OTUs. Our estimates, based on a worldwide survey, are 26 times lower than previous estimates (i.e. ~ 6 000 OTUs) for a subset of sampling stations from the same *Tara* Oceans Expedition (de Vargas *et al.*, 2015), yet twice more than the number of extant collodarian species described to date (i.e. 95 species; Dr. N. Suzuki, pers. comm.). Although some erroneous OTUs might remain in our dataset, we are confident that we are approaching the actual collodarian diversity, stressing the requirement for taxon specific delineation of sequences abundance and clustering thresholds to limit potential diversity overestimations from environmental surveys analyses (Brown *et al.*, 2015). In the case of Collodaria, qPCR estimations of 18S rRNA gene copy

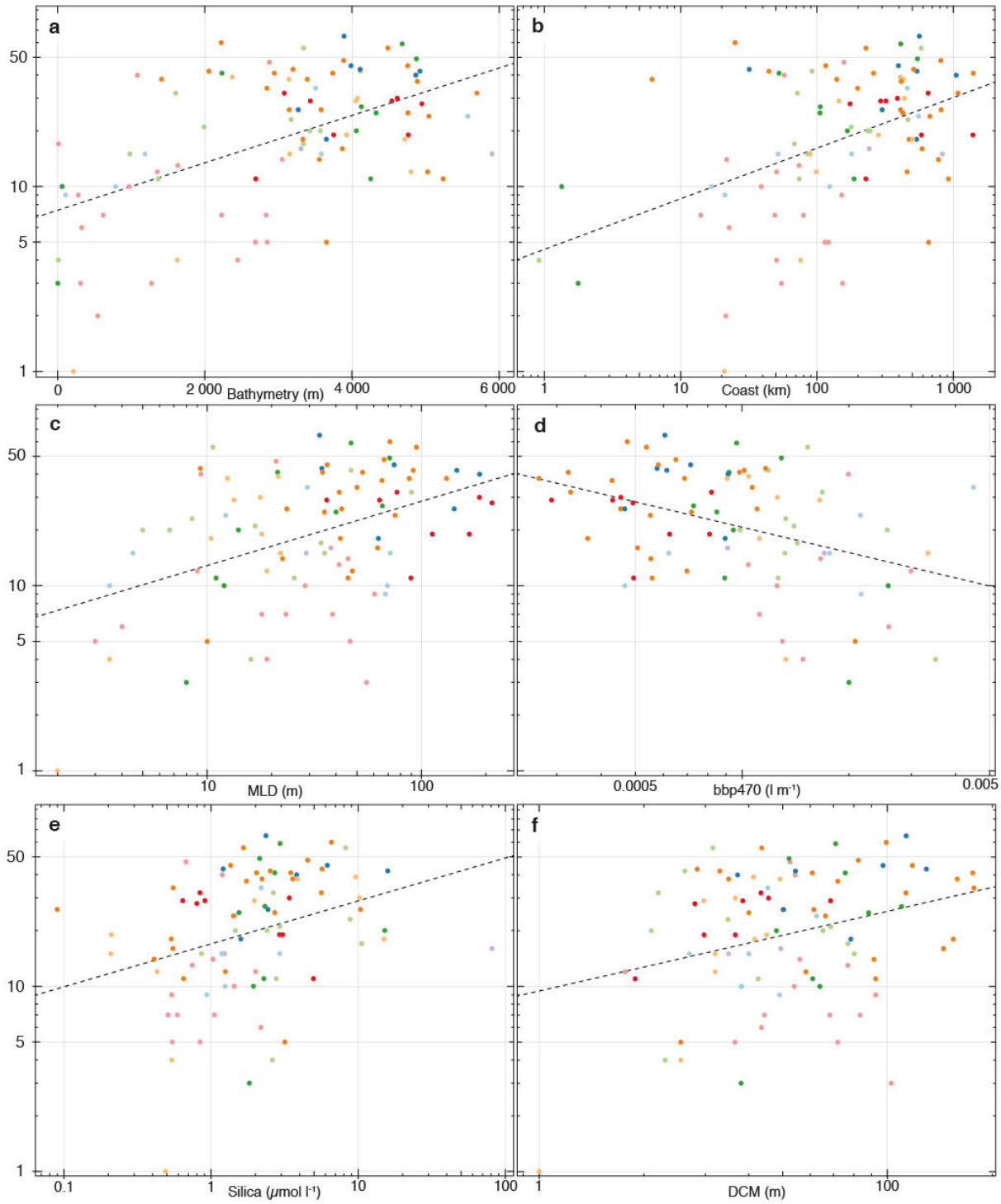

Figure 6 Correlations between the collodarian diversity (log of OTU richness) and mean environmental variables across the *Tara* Oceans sampling stations (linear regression lines are displayed). Each sampling station (dot) is coloured according to one of the Longhurst Biome. (**a**) Relation with the bathymetry (m). (**b**) Relation with the log of the distance to the coast (km). (**c**) Relation with the log of the mixed layer depth (DCM) (m). (**d**) Relation with the log of the backscattering coefficient of particules, 470 nm (bbp470) (l m^{-1}). (**e**) Relation with the log of the silica concentration (µmol m^{-1}). (**f**) Relation with the log of the deep chlorophyll maximum (DCM) (m).

number demonstrated a major difference between solitary and colonial forms (Table 1), two forms that cannot currently be differentiated based on the 18S and 28S rRNA taxonomic genes marker resolution (Biard *et al.*, 2015). In this regards, estimations of central capsule

abundance from sequence abundance will be biased until we are able to separate solitary from colonial forms based on molecular signature, which could potentially be resolved using more variable gene marker such as mitochondrial marker (Leray *et al.*, 2013).

Insight into the biodiversity and ecology of collodarian

Taxonomic assignment of the 230 OTUs based on a recent morpho-molecular reference framework for Collodaria (Biard *et al.*, 2015) did not reveal new lineages represented by environmental sequences only at the family level. At finer taxonomic resolution, a few dominant OTUs, assigned mostly to the Collosphaeridae and Sphaerozoidae, were consistently dominant throughout the different sampling stations suggesting that, besides Collophidiidae, Collodaria are likely to be cosmopolitan organisms (Figures 4b and 5). These observations are consistent with a previous examination of collodarian biodiversity distribution assessed from diverse locations, suggesting that collodarian species can be divided in three groups: 1) widely distributed species typically belonging to the Collosphaeridae clades A4, A5 and A6 or the Sphaerozoidae clade C7, 2) tropical distributed species such as clades A3, B1 or C9, confined to the surface waters and 3) endemics species, absent from any existing morpho-molecular databases (Strelkov and Reshetnyak, 1971; Biard *et al.*, 2015). The clades included in the first category were consistently found being the most abundant throughout the different sampling stations of the present dataset (Figures 4b and 5). We did find strong evidence suggesting the presence of endemic clades even though we recorded several 'rare' OTUs being present in few sampling stations (Figure 4b). Despite our extensive geographical coverage, most of the stations sampled were located in tropical and temperate zones, with only a few coastal conditions and a limited number of polar sampling stations (Supplementary Figure S2). In this respect, we cannot exclude that our sampling might have missed a number of ecological niches where additional collodarian taxa could have been found.

The Collophidiidae family, with its unique genus *Collophidium* (Biard *et al.*, 2015), were rather rarely encountered across the sampling stations investigated in our study (Figures 4a and 5), which were all restricted to the shallow photic layers of the oceans (Pesant *et al.*, 2015). This sampling strategy might have introduced a bias against this family as assignation of environmental sequences, mostly obtained from previous clone libraries surveys, showed that the Collophidiidae were consistently the dominant collodarian family in deep-water samples (Biard *et al.*, 2015). This observation has been confirmed by a recent study from which a substantial number of metabarcodes assigned to the genus *Collophidium* were extracted from deep-water samples (3 000 – 4 000 m) acquired worldwide (Pernice *et al.*, 2015). Explore further this deep-dwelling collodarian community might radically change our understanding of the ecology, diversity and importance of Collodaria in the global oceans.

The observed decrease of collodarian richness towards the high latitudes (Figure 3a) is consistent with previous analyses (Strelkov and Reshetnyak, 1971), but also with other latitudinal trends observed for radiolarians (Boltovskoy *et al.*, 2010) or different marine organisms such as Tintinids or copepods (Dolan *et al.*, 2006; Rombouts *et al.*, 2009). When considering biogeochemical regions defined by Longhurst (Longhurst, 2010), we consistently observed a higher contribution of sequences affiliated to Sphaerozoidae (species mostly lacking silicified structure; Biard *et al.*, 2015) in low-diversity coastal biomes whereas

Collosphaeridae (skeleton-bearing taxa; Biard *et al.*, 2015) were the dominant collodarians in the more diverse open ocean biomes (Figures 3b and 5). Similar to our observations, numerous surface sediment records suggested that Collosphaeridae are likely to be encountered in open-ocean waters (Boltovskoy *et al.*, 2010), but the lack of fossil record for spicule-bearing and naked Collodaria prevent accurate testing of this distribution pattern.

Links between collodarian diversity and environmental variables

Testing the relationships between the collodarian OTU richness, within the different clades or families, with the 14 environmental variables available did not provide any significant correlations (Supplementary Figure S4) but significant relationships were found when considering the total collodarian OTU richness instead (Figure 6). The observed patterns of increasing or decreasing diversity suggest that all three families (with the exception of the Collophidiidae largely under-represented) displayed the same variation for the variables considered and that none of the significant variability affected preferentially one of the families.

Overall, both the bathymetry and the distance from the coast of each sampling station revealed the highest correlation with the OTU richness (Figures 6a and 6b), indicating that the collodarian diversity is likely to increase towards more oceanic conditions, as it was previously suggested (Figure 3b; Swanberg, 1979). We also found a positive correlation between the water column stratification (estimated *via* the mixed-layer depth) suggesting that the collodarian diversity increase for an enhanced vertical stratification of the ocean (Figure 6c). Although the extent of the MLD is variable over seasons, with deeper stratification in winter and a shallow MLD in summer, its extent in tropical regions is rather stable over time, with a deep MLD (de Boyer Montégut *et al.*, 2004). In the present study, most of the samples were collected in tropical regions (Supplementary Figure S2) with deep MLD suggesting an increasing diversity toward tropical and oligotrophic regions where Collodaria are known to be abundant (Dennett *et al.*, 2002; Biard *et al.*, submitted). Such pattern was strengthens by the significant increase of diversity with decreasing Particulate Organic Carbon POC and increasing depth of the chlorophyll maximum DCM. We finally found no significant correlation between OTU richness and sea temperature (Supplementary Table S4) whereas it has been suggested that the collodarian diversity increased towards warmer waters (Strelkov and Reshetnyak, 1971). However, our sampling coverage provide a narrow range of temperature (interquartile range = 7°C, between 18°C and 25°C) and thus might limit the significance of temperature in explaining diversity patterns observed in this narrow inter-tropical regions.

Although the concentrations of silica were rather low over the sampling stations and typical of tropical waters, the overall positive correlation between silica concentration and collodarian diversity (Figure 6e) was unexpected as most Collodaria typically lack silicified structures (i.e. spicules or skeleton). The analysis of OTU composition indicated that the diversity of the three collodarian families increased with higher silica concentration while we could have expect a domination of naked Sphaerozoidae (e.g. *Collozoum* spp.) in low silica concentration waters (Supplementary Figure S4). The decrease of radiolarian skeleton weight with decreasing silica availability in surface waters has been previously suggested through analysis of marine sediments from the Cenozoic (i.e. <60 m.y.; Lazarus *et al.*, 2009).This

could however indicate that with increasing silica concentration, a large diversity of form, in particular silicified collodarian (i.e. spicules and skeleton-bearing species) could be encountered.

The cosmopolitan distribution of collodarian families and clades observed in the present study provide insights into the ecology of Collodaria and suggested these protists have adapted to various environmental conditions. Although we recovered six significant relationships with different abiotic parameters, other processes might be considered to fully understand the distribution of collodarian diversity, such as the influence of temporal scales as it has been proved to have potential impacts on plankton diversity (Rombouts *et al.*, 2010; Egge *et al.*, 2015), or including biotic variables such as predation (Shurin, 2001) or photosymbiosis (Biard *et al.*, submitted).

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Supplementary Table Legends

Supplementary Table S1 List of rDNA copy number from different eukaryotic groups.

Organism	Copies per cell	Cell length (μm)	Reference
Chlorophyta			
Bathycoccus prasinos	12	1.5	Zhu *et al.*, 2005
Chlamydomonas concordia	450	7	Zhu *et al.*, 2005
Micromonas pusilla	5	1.5	Zhu *et al.*, 2005
Micromonas pusilla	5	1.5	Zhu *et al.*, 2005
Ostreococcus tauri	4	0.8	Zhu *et al.*, 2005
Prasinococcus capsulatus	2	3	Zhu *et al.*, 2005
Tetraselmis sp.	34	8	Zhu *et al.*, 2005
Ciliates			
Cryptocaryon irritans	3 415	400	Taniguchi *et al.*, 2011
Epistylis sp.	64 865	145	Gong *et al.*, 2013
Epistylis sp.	88 161	145	Gong *et al.*, 2013
Favella sp.	46 498	220	Gong *et al.*, 2013
Oxytricha nova	100 000	150	Prescott, 1994
Pseudotontonia sp.	172 889	120	Gong *et al.*, 2013
Tetrahymena thermophila	9 000	50	Kapler 1993
Tintinnopsis sp.	126 372	195	Gong *et al.*, 2013
Strombidinopsis sp.	30 247	100	Gong *et al.*, 2013
Strombidium sp.	34 647	45	Gong *et al.*, 2013
Stylonychia lemnae	200 000	230	Heyse *et al.*, 2010
Vorticella sp.	160 000	100	Liu and Gong, 2012
Vorticella sp.1	161 355	105	Gong *et al.*, 2013
Vorticella sp.2	315 786	105	Gong *et al.*, 2013
Vorticella sp.3	99 376	100	Gong *et al.*, 2013
Vorticella sp.3	61 226	100	Gong *et al.*, 2013
Vorticella sp.5	82 194	100	Gong *et al.*, 2013
Zoothamnium sp.1	40 675	35	Gong *et al.*, 2013
Zoothamnium sp.2	3 385	35	Gong *et al.*, 2013
Collodaria			
Sphaerozoum fuscum	14 933	118	This study
Sphaerozoum sp.	70 556	130	This study
Sphaerozoum fuscum	23 275	112	This study
Sphaerozoum fuscum	32 895	96	This study
Sphaerozoum fuscum	18 470	104	This study
Sphaerozoum fuscum	41 374	112	This study
Collozoum pelagicum	32 977	89	This study
Collozoum pelagicum	74 834	87	This study
Collozoum pelagicum	47 950	68	This study
Collozoum pelagicum	28 755	77	This study
Collozoum pelagicum	60 450	85	This study
Collozoum pelagicum	25 765	86	This study
Collozoum pelagicum	48 009	73	This study
Procyttarium primordialis	1 790	661	This study
Procyttarium primordialis	5 355	374	This study
Procyttarium primordialis	13 250	370	This study
Procyttarium primordialis	3 980	370	This study
Copepods			
Calanus finmarchicus	153 000 000*	2 840	Wyngaard *et al.*, 1995
Calanus glacialis	335 000 000*	3 970	Wyngaard *et al.*, 1995
Mesocyclops edax	8 400 000*	1 500	Wyngaard *et al.*, 1995
Mesocyclops edax	7 300 000*	1 500	Wyngaard *et al.*, 1995
Diatoms			
Coscinodiscus wailesii	33 689	200	Godhe *et al.*, 2008
Cylindrotheca closterium	484	67	Godhe *et al.*, 2008

Ditylum brightwellii	36 896	69	Godhe *et al.*, 2008
Extubocellulus spinifer	150	4	Godhe *et al.*, 2008
Nitzschia closterium	199	20	Zhu *et al.*, 2005
Phaeodactylum tricornicum	88	24	Godhe *et al.*, 2008
Skeletonema marinoi	61	9	Godhe *et al.*, 2008
Thalassioria sp.	636	15	Zhu *et al.*, 2005
Thalassioria weisflogii	49	10	Zhu *et al.*, 2005
Dictyochophyceae			
Mesopedinella arctica	33	8	Zhu *et al.*, 2005
Dinoflagellates			
Akashiwo sanguineum	30 545	50	Zhu *et al.*, 2005
Alexandrium andersonii	1 603	23	Godhe *et al.*, 2008
Amphidinium carterae	840	15	Zhu *et al.*, 2005
Gymnodinium chlorophorum	1 673	17	Godhe *et al.*, 2008
Heterocapsa triquetra	4 872	19	Godhe *et al.*, 2008
Lingulodinium polyedrum	12 812	38	Godhe *et al.*, 2008
Peridinium faeroense	1 057	28	Godhe *et al.*, 2008
Prorocentrum micans	4 996	49	Godhe *et al.*, 2008
Prorocentrum minimum	1 432	20	Zhu *et al.*, 2005
Prorocentrum nux	143	10	Zhu *et al.*, 2005
Prorocentrum lima	10 086	42	Godhe *et al.*, 2008
Protoceratium reticulatum	6 644	35	Godhe *et al.*, 2008
Scrippsiella trochoidea	4 112	28	Godhe *et al.*, 2008
Foraminifera			
Allogromia laticollaris	40 000	175	Weber and Pawlowski, 2013
Allogromia laticollaris	30 000	175	Weber and Pawlowski, 2013
Bolivina variabilis	5 000	175	Weber and Pawlowski, 2013
Bolivina variabilis	10,000	175	Weber and Pawlowski, 2013
Rosalina sp.	10 000	175	Weber and Pawlowski, 2013
Haptophyta			
Emiliania huxleyi	4	3	Zhu *et al.*, 2005
Rhodophyta			
Rhodomonas salina	204	10	Zhu *et al.*, 2005
Stramenopiles			
MAST-4	30	2.5	Rodriguez-Martinez *et al.*, 2009
Nannochloropsis salina	1	2	Zhu *et al.*, 2005

* The number of rDNA copy for Copepods was multiply by the approximated number of cells within one single organism (~10 000 cells; McLaren and Marcogliese, 1983).

Supplementary Table S2 List of the closest un-/cultured matches of BLAST against GenBank and manually assigned according to Biard *et al.* (2015).

OTU ID	Sequences (n)	Closest match	Identity	Query cover	Clade ID
Collosphaeridae (Clade A)					
15	7 386 683	Uncultured Eukaryote (AY256280)	98.4	97	A
28	5 314 033	*Siphonosphaera cyathina* (AF091145)	85.38	100	A5
26	4 202 996	*Collosphaera tuberosa* (KR058209)	91.3	88	A6
328	436 345	*Collosphaera tuberosa* (KR058209)	89.57	88	A
266	402 866	Uncultured Eukaryote (AY256280)	96	97	A
551	372 482	*Acrosphaera* sp. (AF091148)	94.57	100	A4
576	167 107	Uncultured Eukaryote (AY256280)	96.8	97	A
479	158 947	*Collosphaera tuberosa* (KR058209)	90.43	88	A6
5256	6 154	*Collosphaera tuberosa* (KR058209)	91.3	88	A6
7730	2 536	*Collosphaera tuberosa* (KR058209)	88.7	88	A6
7595	1 738	*Collosphaera tuberosa* (KR058209)	90.43	88	A6
Collophidiidae (Clade B)					
192	943 740	Uncultured Eukaryote (AY882496)	99.22	97	B
174	625 004	*Collophidium serpentinum* (AF018162)	87.97	100	B1
321	381 727	Uncultured Eukaryote (GU825111)	89.84	97	B
353	329 606	Uncultured Eukaryote (AY046753)	96.83	96	B
315	292 735	Uncultured Eukaryote (GU823723)	93.75	97	B
310	292 477	Uncultured Eukaryote (AY882496)	97.66	97	B
330	284 198	Uncultured Eukaryote (GU823723)	96.09	97	B
564	201 344	Uncultured Eukaryote (GU825502)	90.15	97	B
511	199 633	Uncultured Eukaryote (GU825067)	100	97	B
605	186 828	Uncultured Eukaryote (GU825111)	100	97	B
493	176 382	Uncultured Eukaryote (AY256259)	100	97	B
495	116 907	Uncultured Eukaryote (GU823723)	100	97	B
973	100 489	Uncultured Eukaryote (AY882496)	96.88	97	B
334	82 075	Uncultured Eukaryote (GU825285)	100	97	B
809	53 461	Uncultured Eukaryote (GU825502)	98.44	97	B
1629	38 728	Uncultured Eukaryote (AY046753)	92.86	96	B
2048	27 765	Uncultured Eukaryote (AY256259)	92.97	97	B
2651	18 010	Uncultured Eukaryote (AY882496)	97.66	97	B
2830	16 400	Uncultured Eukaryote (AY882496)	97.66	97	B
3039	15 411	Uncultured Eukaryote (GU823723)	96.09	97	B
3076	10 792	Uncultured Eukaryote (GU825111)	89.84	97	B
1522	9 535	Uncultured Eukaryote (AY046753)	97.62	96	B
3814	8 512	Uncultured Eukaryote (GU825502)	96.09	97	B
3396	6 875	Uncultured Eukaryote (GU825111)	96.88	97	B
1118	6 798	Uncultured Eukaryote (AY046753)	99.21	96	B
2965	5 796	Uncultured Eukaryote (AY256259)	94.53	97	B
5340	5 524	Uncultured Eukaryote (GU825111)	92.19	97	B
2739	4 026	Uncultured Eukaryote (AY256259)	89.84	97	B
4946	2 858	Uncultured Eukaryote (AY882496)	99.22	97	B
6674	2 408	Uncultured Eukaryote (GU825111)	91.41	97	B
4106	2 247	Uncultured Eukaryote (GU825067)	96.09	97	B
8468	2 161	Uncultured Eukaryote (GU823723)	92.19	97	B
7002	1 865	Uncultured Eukaryote (AY256259)	96.09	97	B
6408	1 837	Uncultured Eukaryote (AY882496)	97.66	97	B
6295	1 698	Uncultured Eukaryote (AY882496)	96.09	97	B
8555	1 565	Uncultured Eukaryote (GU825502)	90.7	97	B
7278	1 283	Uncultured Eukaryote (GU825067)	99.24	99	B
8007	1 279	Uncultured Eukaryote (GU825013)	97.66	97	B
9108	1 186	Uncultured Eukaryote (GU825111)	91.41	97	B
12206	1 148	Uncultured Eukaryote (AY882496)	96.88	97	B
12460	1 023	Uncultured Eukaryote (GU825111)	89.84	97	B

Sphaerozoidae (Clade C)					
22	12 561 422	*Collozoum inerme* (AY266295)	98.47	100	C7
55	2 551 556	*Collozoum amoeboides* (AB613239)	96.9	98	C
171	2 021 272	*Sphaerozoum punctatum* (AB613248)	96.95	100	C
150	1 714 462	*Rhaphidozoum acuferum* (AF091147)	94.66	100	C
292	450 989	*Sphaerozoum punctatum* (AB613248)	92.31	100	C
597	273 772	*Collozoum inerme* (AY266295)	96.18	100	C7
369	207 206	*Sphaerozoum punctatum* (AB613248)	94.62	100	C
609	169 778	*Collozoum inerme* (AY266295)	89.55	100	C7
611	159 557	*Sphaerozoum punctatum* (AB613248)	96.21	100	C
847	131 191	*Sphaerozoum punctatum* (AB613248)	96.21	100	C
837	112 816	*Sphaerozoum punctatum* (AB613248)	93.85	99	C
1027	85 515	*Rhaphidozoum acuferum* (AF091147)	94.66	100	C
1437	62 041	*Collozoum amoeboides* (AB613239)	96.95	100	C
1182	61 970	*Rhaphidozoum acuferum* (AF091147)	94.66	100	C
1606	43 764	*Collozoum pelagicum* (AF091146)	96.95	100	C10
1195	38 724	*Rhaphidozoum acuferum* (AF091147)	93.89	100	C
1835	34 884	*Rhaphidozoum acuferum* (AF091147)	90.77	99	C
2271	29 887	*Rhaphidozoum acuferum* (AF091147)	93.89	100	C
1328	27 555	*Collozoum inerme* (AY266295)	95.42	100	C7
2273	21 859	*Collozoum inerme* (AY266295)	96.95	100	C7
2055	16 062	*Collozoum inerme* (AY266295)	96.95	100	C7
2893	15 199	*Collozoum inerme* (AY266295)	96.95	100	C7
2099	15 043	*Collozoum inerme* (AY266295)	94.78	100	C7
2141	14 130	*Sphaerozoum punctatum* (AB613248)	96.95	100	C
2288	12 943	*Collozoum inerme* (AY266295)	97.71	100	C7
3288	9 212	*Collozoum amoeboides* (AB613239)	96.95	100	C
2520	8 760	*Collozoum amoeboides* (AB613239)	96.95	100	C
3653	7 776	*Sphaerozoum punctatum* (AB613248)	96.18	100	C
6065	4 088	*Collozoum amoeboides* (AB613239)	96.95	100	C
5525	3 958	*Sphaerozoum punctatum* (AB613248)	96.21	100	C
2641	2 741	*Collozoum inerme* (AY266295)	95.42	100	C7
5113	2 705	*Collozoum amoeboides* (AB613239)	95.35	98	C
5989	2 389	*Sphaerozoum punctatum* (AB613248)	98.45	98	C
9187	2 012	*Sphaerozoum punctatum* (AB613248)	96.21	100	C
3341	1 165	*Collozoum inerme* (AY266295)	96.95	100	C7
12529	1 034	*Collozoum inerme* (AY266295)	93.89	100	C7
11273	1 025	*Rhaphidozoum acuferum* (AF091147)	90.84	100	C

Supplementary Table S3 Relative abundance of rDNA metabarcodes across the *Tara* Ocean sampling stations.

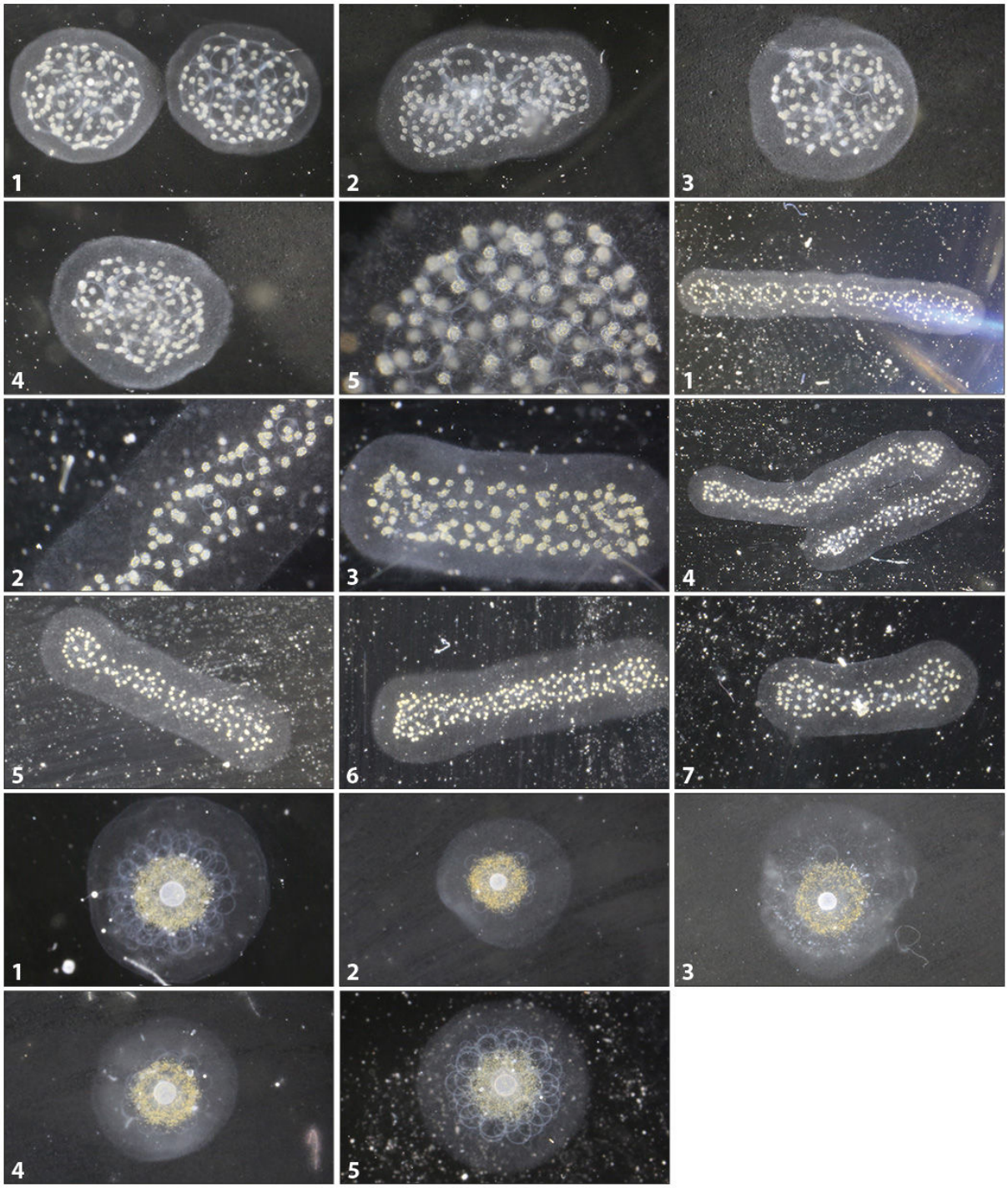
Station	Collosphaeridae					Collophidiidae				Sphaerozoidae					
	A3	A4	A5	A6	A	B1	B2	B	C1	C6	C7	C10	C11	C	C sp.1	C sp.2
4	0	1 025	0	0	0	0	0	1 817	8 884	0	535 049	2 499	0	1 040	12 441	0
5	8 953	0	0	10 158	0	0	0	0	0	0	0	0	0	0	0	0
7	0	1 105	0	0	0	0	0	1 290	0	0	129 882	3 378	0	27 258	0	0
8	0	0	0	0	2 504	0	0	0	0	0	21 991	3 292	0	11 148	0	0
9	685 246	82 045	13 260	35 797	9 595	10 806	33 267	21 130	38 978	0	202 880	26 663	0	12 718	8 062	0
10	0	0	0	0	1 368	0	0	7 203	4 709	0	74 528	0	0	8 233	0	2 597
11	0	109 997	6 676	5 224	160 085	0	0	0	79 128	0	127 913	25 120	0	6 539	1 485	0
12	0	0	0	0	0	0	0	17 305	0	0	61 738	0	0	0	0	0
17	0	0	0	0	9 921	0	0	0	0	0	2 165	0	0	0	0	0
18	0	69 622	0	94 548	56 678	0	0	0	57 418	0	22 084	0	0	0	0	0
19	0	3 021	0	41 025	73 163	0	0	0	0	0	0	0	0	0	0	0
20	0	9 170	0	273 156	31 772	0	0	0	1 113	0	8 912	0	2 176	0	0	0
21	0	27 807	0	199 337	58 275	0	0	0	1 021	0	1 075	0	0	0	0	0
22	0	506 028	117 830	0	423 580	0	0	2 127	100 908	0	15 948	2 922	0	0	0	0
23	7 204	1 126 519	30 409	7 584	576 171	13 253	0	24 404	315 854	0	111 859	0	0	471 337	455 256	0
24	0	983 722	0	47 623	53 319	0	0	0	7 646	0	0	0	0	0	0	0
25	0	1 795 833	0	429 872	507 182	0	0	0	49 185	0	45 639	0	0	0	0	3 270
26	0	487 196	6 377	13 829	379 952	0	0	0	112 117	0	9 979	2 278	0	0	0	5 679
30	0	46 707	1 529	8 008	15 129	0	0	2 171	39 140	0	50 542	0	0	10 343	5 227	0
32	29 683	10 099	0	16 774	0	17 062	4 814	1 458	10 553	0	26 970	2222	0	0	0	0
34	0	5 268	0	7 470	0	0	0	0	7 986	0	11 382	0	0	9 078	7 082	6 485
36	0	4 884	0	0	0	0	1 441	3 118	76 197	0	2 814 453	0	0	101 462	0	0
38	33 851	1 187 504	21 786	78 745	108 623	104 808	1 123	500 775	12 495	0	153 643	3 087	0	102 044	106 849	0
39	3 920	356 792	2 019	2 230	11 127	2 995	1 390	1 786	47 085	0	159 837	0	0	2 508	3 418	0
41	367 020	43 534	2 988	0	0	0	42 661	13 933	2 330	0	931 277	162 126	0	31 566	24 189	0
42	123 071	3 922 565	10 828	634 009	83 478	17 863	0	152 171	60 076	0	61 822	3 024	1 636	55 760	50 172	0
43	0	846 122	1 241	0	0	0	0	0	0	0	1 096	0	0	0	0	0
45	10 460	3 201 173	132 205	1 262	0	0	0	1148	507340	0	29 533	0	0	77 934	0	0
46	0	500 218	0	0	0	0	0	12 410	0	0	482 708	0	0	147 006	0	0
47	0	89 907	1 481	245 161	0	0	0	0	66 132	0	12 787	0	0	23 347	0	0
50	215 103	80 960	50 641	480 447	0	10 794	0	0	1 775	0	6 131	0	8024	24 012	2 310	6 413
51	4 786	35 566	39 478	273 553	0	14 057	1 576	1 827	32 016	0	70 178	0	0	80 343	3 212	2 655
52	1 862 560	432 911	850 613	456 741	9 769	48 683	549 251	26 701	65 043	0	40 880	0	5202	176 296	122 394	0
53	0	3 971	4 222	0	0	0	0	1 413	21 848	0	82 656	0	0	36 304	9 192	0
54	0	20 137	0	0	0	0	0	0	27 950	0	6 556	0	0	7 973	0	0
58	27 067	32 988	55 841	87 478	1 180	0	0	9 080	49 520	0	19 721	0	0	19 312	0	0
64	383 107	142 418	16 881	141 420	0	347 398	49 676	29 580	2 267 603	0	364 194	37 970	0	25 154	86 177	0
65	202 033	509 267	39 099	55 629	3 587	2 799	9 256	73 980	308 131	0	229 977	0	3341	82 460	12 748	0
66	2 215	45 488	26 842	3 267	76 534	10 920	0	17 376	15 975	0	21 605	0	0	122 858	116 307	0
68	17 717	37 857	16 957	19 602	1 792 247	230 528	0	150 379	58 219	0	326 040	0	0	94 129	302 996	0
70	380 887	19 169	507 158	74 642	407 241	19 302	0	38 904	66 554	0	154 450	0	0	1 478	23 113	0
71	67 569	1 250	26 372	132 370	18 187	9 122	1 938	0	2 357	0	8 310	0	0	0	0	0
72	122 736	131 593	963 471	1 472 296	234 946	0	0	3 223	51 447	0	921 348	5 550	0	19 772	9 102	0
76	727 182	2 268 841	143 441	415 723	24 085	8 232	0	0	158 363	0	110 426	2 776	0	877 397	51 378	0
78	1 055 983	723 872	157 459	30 046	1 073	87 544	2 658	171 817	53 374	0	460 097	0	0	554 669	566 905	0
80	16 308	42 697	122 670	59 217	0	2 017	0	0	6 484	0	24 087	0	1122	73 994	34 272	0
81	33 084	85 508	24 913	18 581	0	0	0	0	7 915	0	36 995	0	0	1 140	3 131	0

82	7 674	186 601	70 250	5 391	1 334	9 131	1 134	52 734	7 532	0	23 364	0	0	393 414	386 558	0		
83	0	0	10 120	9 015	0	0	0	9 335	12 998	0	47 429	0	1361	2 498	0	0		
85	2 865	401 902	9 726	3 317	0	1 801	0	8 838	0	0	3 521	0	0	59 750	60 714	0		
90	0	577 975	95 226	0	117 947	0	0	0	7 338	0	194 156	0	0	156 764	5 619	0		
91	0	65 716	4 495	0	0	0	0	4 232	100 901	0	182 166	0	0	32 748	22 567	0		
92	0	0	0	0	0	0	0	0	16 493	0	0	0	0	0	0	0		
93	0	268 704	8 648	0	0	2 514	0	6 800	405 237	0	110 514	1046	0	143 415	0	0		
94	22 009	267 086	0	2 338	0	26 338	0	1 198	441 154	0	370 833	42794	0	149 726	59 808	0		
95	0	185 958	2 804	3 179	90 527	1 799	0	0	3 593	0	51 023	0	0	4 806	4 780	0		
96	58 275	45 938	74 371	138 671	0	8 705	0	0	1 170	0	198 229	1391	0	29 826	26 408	0		
97	377 734	218 654	311 011	147 839	0	30 007	0	22 922	23 812	0	21 498	1576	3083	3 186	0	0		
98	1 103 328	208 920	1 439 228	80 891	58 445	34 390	1 319	103 381	10 925	0	15 394	0	2554	238 470	182 142	0		
99	2 711	2 734	5 412	281 454	0	12 062	0	0	1 161	0	37 144	0	0	43 426	41 866	0		
100	14 214	128 697	11 931	1 248 956	0	37 436	105 379	17 952	1344 971	0	310 524	1409	0	169 150	83 430	0		
102	0	3 904	448 885	248 368	1 862	0	0	36 254	292 946	0	1 579 902	36262	0	127 033	6 698	12 486		
106	82 671	653 461	18 115	777 430	0	0	0	86 631	54 323	0	198 697	1250	0	262 980	6 457	4 202		
109	16 243	709 561	131 405	148 006	2 667	1 717	0	123 929	55 807	0	69 675	32343	0	15 114	0	0		
110	3 713	2 224 725	160 581	293 275	23 566	2 745	12 272	269 454	35 225	0	146 566	51751	0	7 497	0	0		
111	95 882	191 636	84 769	1 379 832	121 991	2 074	104 833	266 113	376 960	0	266 678	0	0	112 086	4 033	0		
112	92 369	239 242	253 616	286 793	0	17 663	0	0	70 998	2 117	220 438	0	0	26 950	7 033	0		
122	113 973	186 177	568 271	1 693 054	47 287	11 041	10 328	4 306	139 909	0	577 674	65276	0	379 050	14 899	58 326		
123	303 573	93 014	181 749	541 856	0	2 412	0	6 694	695 587	0	109 225	7033	0	316 427	18 420	3 926		
124	276 178	160 648	567 146	419 190	16 041	5 705	0	0	61 832	0	170 841	8187	0	330 787	4 305	7 954		
125	384 930	513 184	674 517	3 255 662	192 447	1 653	0	31 755	598 982	0	845 402	11005	0	235 661	33 784	387 668		
126	16 042	67 548	1 048	135 455	0	0	0	1 494	19 754	0	64 575	0	0	0	0	0		
127	606 965	275 876	67 253	242 953	1 482	0	24 392	2 515	106 495	0	235 390	0	0	402 633	0	73 125		
128	6 749	62 020	176 658	404 509	3 080	0	12 604	5 142	12 952	0	55 222	45488	0	92 074	0	0		
129	90 544	27 412	128 459	203 008	105 347	0	1 045	1 963	19 023	0	5 048	0	0	6 619	0	0		
130	226 010	0	0	9 473	0	7 236	0	0	9 787	0	0	0	0	4 539	0	0		
131	47 779	471 396	1 811 806	1 936 703	0	3 304	43 580	55 165	35 250	0	24 485	1435	5295	53 402	2 111	1 688		
132	140 902	12 306	53 317	4 520	0	435 802	69 207	2 799	22 502	0	42 186	0	0	57 859	5 236	0		
133	1 121	34 466	0	22 231	0	0	0	43 223	169 062	0	149 826	0	0	5 372	10 215	0		
135	1 182	42 724	0	5 428	18 411	25 935	2 206	780 741	286 910	0	1 252 590	0	0	52 582	1 771	0		
138	296 092	54 231	3 579	264 034	0	19 453	8 269	135 746	164 925	0	75 272	0	0	27 515	0	12 338		
139	0	0	0	17 780	0	0	0	44 753	0	0	4 894	0	0	2 269	0	0		
140	2 687	0	0	75 657	0	0	0	0	0	0	9 428	0	0	26 648	0	0		
141	0	10 357	22 243	53 823	19 617	0	4 709	0	36 079	0	267 075	0	0	51 172	2 951	0		
142	1 110	1 027	92 320	19 206	0	2 886	2 602	3 856	226 417	1 186	313 252	0	3001	270 758	26 623	0		
143	0	1 066	31 446	24 810	0	0	0	24 168	23 912	0	140 705	0	2448	22 399	0	0		
144	2 163	0	213 053	11 467	3 445	0	1 045	106 942	15 679	0	36 283	0	0	0	0	0		
145	0	7 396	52 578	0	0	0	0	2 390	16 633	0	346 017	0	0	0	0	0		
146	0	1 289	1 764 070	14 485	45 428	4 041	0	38 761	72 193	0	542 558	0	0	13 010	0	0		
147	0	2 483	1 493 010	17 538	28 179	43 955	0	23 439	130 297	0	88 038	0	0	8 260	66 096	0		
148	0	56 909	936 317	14 586	183 124	12 030	0	10 367	21 302	0	24 916	0	0	15 473	0	0		
149	0	0	213 782	22 887	181 266	60 258	0	16 297	12 400	0	36 630	0	0	0	0	0		
150	4 388	55 304	422 472	77 497	1 067 016	71 714	0	3 903	15 499	1 057	85 607	0	0	2 611	0	0		
151	8 793	23 283	448 462	115 721	386 799	28 299	0	9 549	11 514	0	54 065	0	16530	55 529	21 86	0		

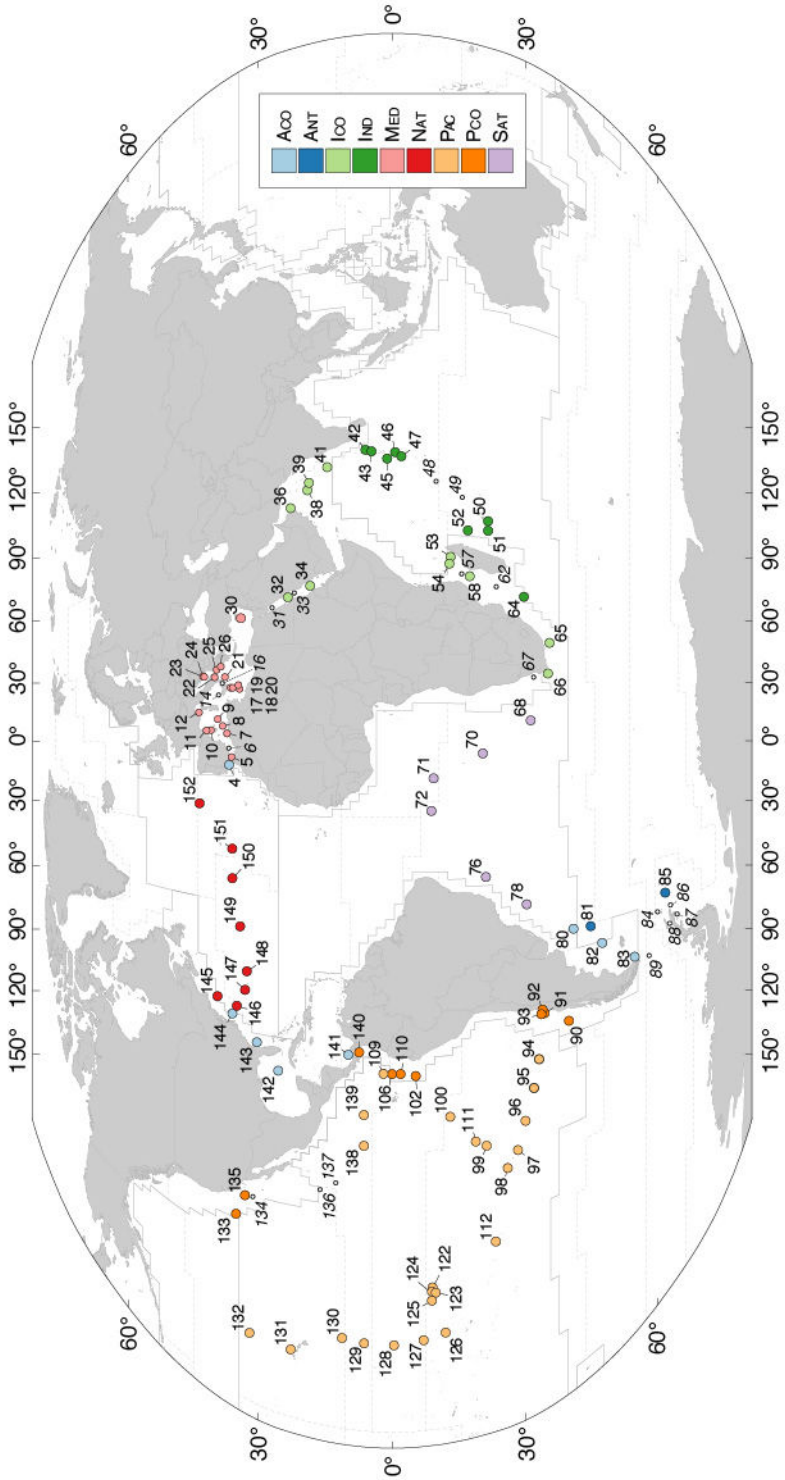
Supplementary Table S4 Correlations (Pearson) between collodarian diversity (log of OTUs richness) and environmental variables (mean of surface and DCM values). *P-value* (ns: *p-value* > .05, one star: *p-value* < .05, two stars: *p-value* < .01, three stars: *p-value* < .001).

Environmental variable	Short name	Correlation coefficient (r)	Degrees of freedom ($n - 2$)
Bathymetry (m)	-	0.5456 ***	93
Coast distance (km)	-	0.4706 ***	92
Mixed-layer depth (m)	MLD	0.4360 ***	90
Backscattering coefficient of particles, 470 nm (l m^{-1})	bbp470	-0.3933 ***	80
Silicate (µmol l^{-1})	$Si(OH)_4$	0.3223 **	86
Depth of chlorophyll maximum (m)	DCM	0.2973 **	79
Phosphate (µmol l^{-1})	PO_4	0.2878 ns	81
Depth of minimum oxygen concentration (m)	Min O_2	0.2529 ns	85
Fluorescence, colored dissolved organic matter (ppb QSE)	fCDOM	0.2099 ns	80
Nitrite (µmol l^{-1})	NO_2	0.1630 ns	74
Salinity	-	-0.0908 ns	90
Mass flux at 150 m derived from particle abundance size spectra (mg m^{-2} d^{-1})	Flux 150 m	0.0674 ns	71
Chlorophyll *a* (mg m^{-3})	Chl *a*	-0.0435 ns	89
Ocean temperature (°C)	Temperature	-0.005 ns	90

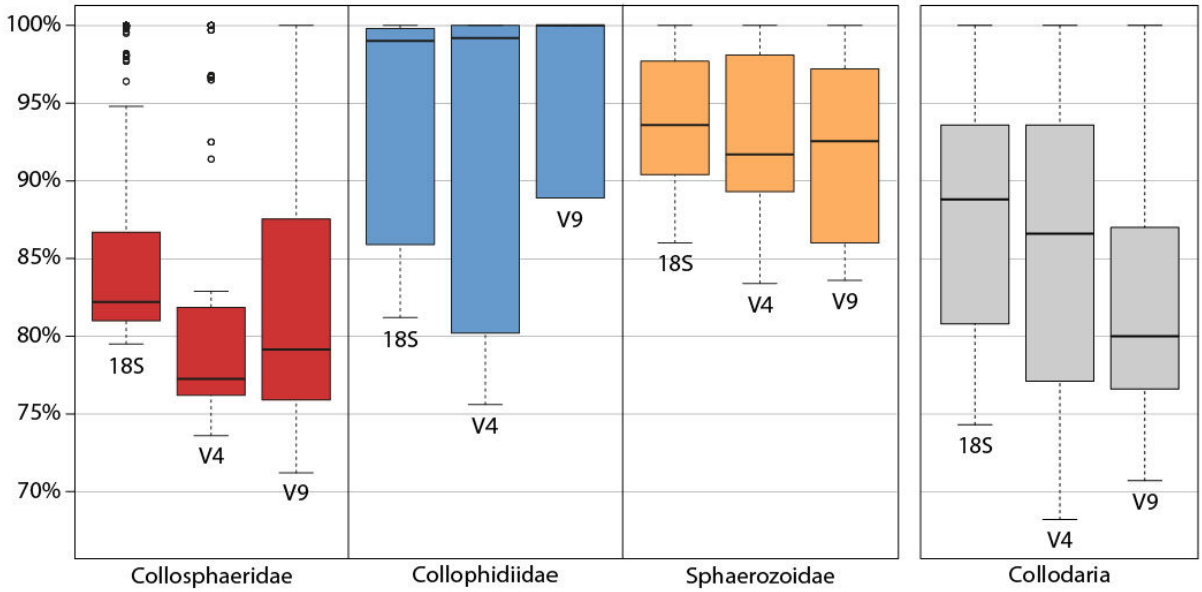
Supplementary Figure S1 Light micrographs of collodarians specimens (species names, clade affiliation and sequence availability are shown in Table 1).



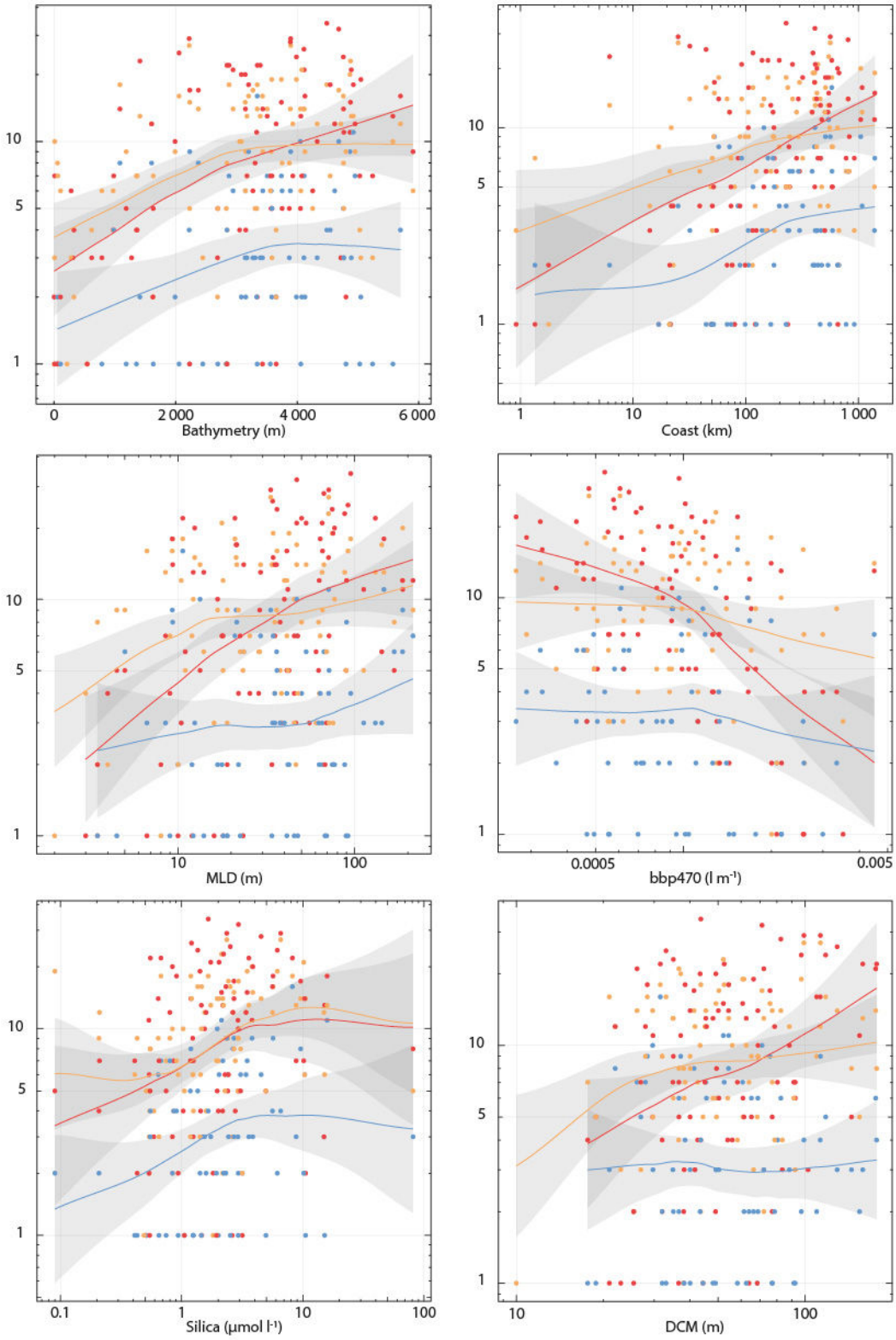
Supplementary Figure S2 Sampling stations of the *Tara* Oceans Expedition used for diversity analyses of the Collodaria. Each station was affiliated to one of Longhurst's Biogeochemical Provinces and assembled in Biomes (Longhurst, 2010). Empty dots with italic number represent the sampling stations lacking collodarian sequences. Map made with Natural Earth data (http://www.naturalearthdata.com).



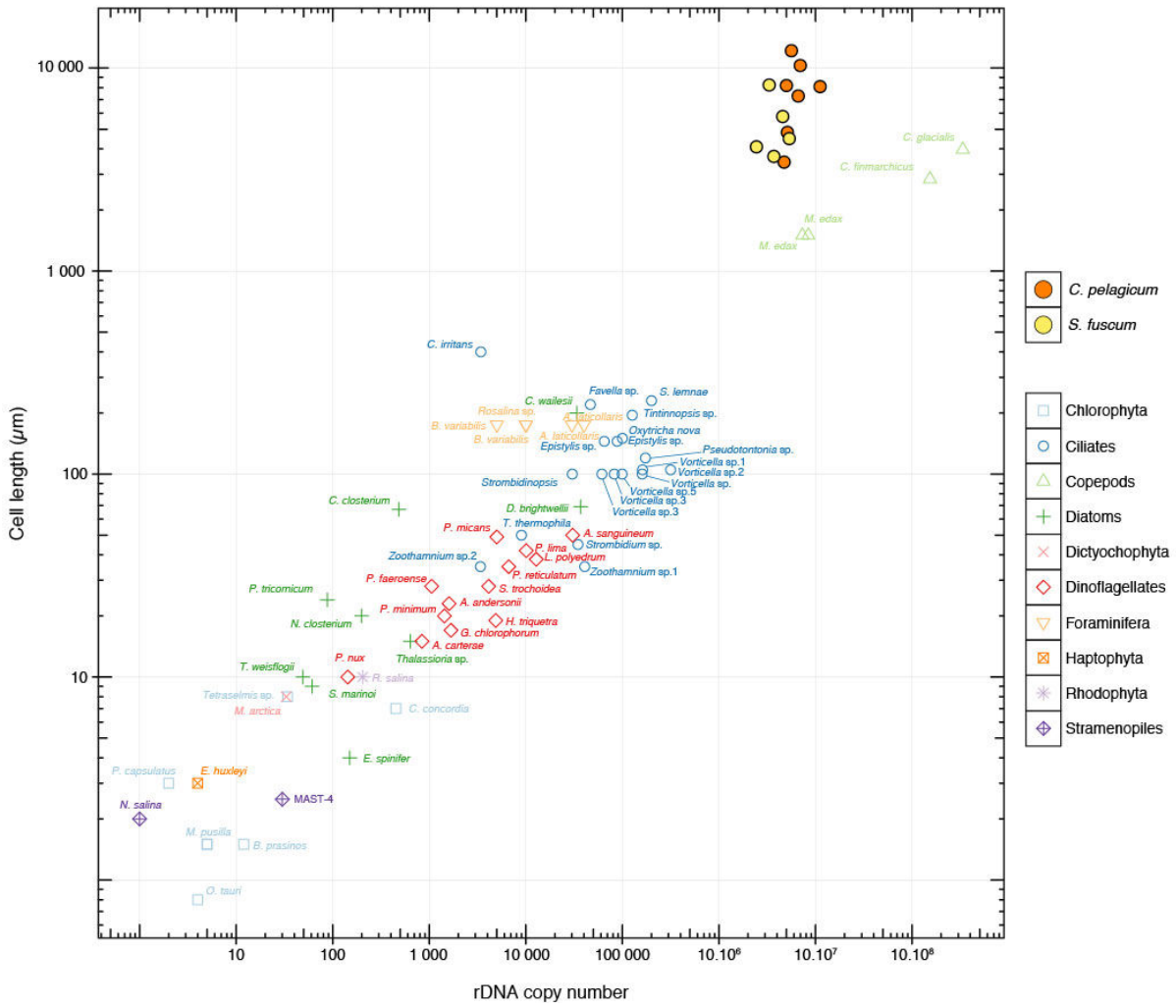
Supplementary Figure S3 Boxplot of interspecific divergence (percent identity score) for the full 18S rRNA gene and the two hypervariables regions V4 and V9. (**a**) Divergence within the three collodarian families. (**b**) Divergence within the Collodaria.



Supplementary Figure S4 Relationships between the three collodarian families (log of OTU richness) and mean environmental variables across the *Tara* Oceans sampling stations. Loess regressions with polynomial fitting were computed to illustrate the relationships. Pale colour area displays a 0.95 confidence interval around the trend.



Supplementary Figure S5 Comparison between the rDNA copy number per cell estimated by quantitative PCR and the cell length across eukaryotic taxa. The different colonial collodarian specimen are represented considering the entire colony (filled circles).



CHAPITRE II-2

Seasonal dynamics of rhizarian communities in the north-western Mediterranean Sea revealed by high-throughput sequencing

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INTRODUCTION

Monitoring the seasonal dynamics of plankton communities provides useful tool to understand the ecology and/or the change in biodiversity structure due to environmental or climate-variability (Perry *et al.* 2004; Hays *et al.* 2005; Richardson 2008). Due to its peculiar nature, a semi-enclosed bay with a deep bathymetry and located on a narrow continental shelf, the Bay of Villefranche-sur-Mer, located in the north-western part of the Ligurian Sea (Mediterranean), is a coastal site with open ocean conditions, providing an useful location to study zooplankton. Historically, time-series investigation on zooplankton communities have been frequently performed in the Bay of Villefranche-sur-Mer, where seasonality and long-term changes of different zooplankton groups have been studied over the last 30 years (Licandro *et al.* 2006; García-Comas *et al.* 2011; Vandromme *et al.* 2011; Romagnan *et al.* 2015; Howes *et al.* 2015). Despite the existence of such a valuable zooplankton time-series, sampled weekly since 1966 (Berline *et al.* 2012), the seasonality of fragile but ecologically important zooplankton taxa has never been investigated specifically.

The Rhizaria, one the main eukaryotic super-group (Burki & Keeling 2014), is a diverse phylum encompassing heterotrophic marine protists, and sub-divided in the Retaria and Cercozoa taxa (Moreira *et al.* 2007). Rhizaria are amoeboid cells, among which some are characterised by complex and delicate skeletons (shells or tests) embracing the cells and made of either calcium carbonate (Foraminifera; Kimoto 2015) or either siliceous and strontium sulfate (Radiolaria; Suzuki & Not 2015). Marine Rhizaria are widespread in the oceans, from polar to oligotrophic regions and from surface photic layers to the deep aphotic layers (Kimoto 2015; Suzuki & Not 2015). Large Rhizaria (>600 µm) have been increasingly recognized as key components of plankton communities, representing 4.5% of the total oceanic biota carbon reservoir in the upper 200 m of the world oceans (Biard *et al.* submitted), and playing important roles in the export of particles to the deep ocean (Lampitt *et al.* 2009). Unfortunately, the impossibility to cultivate them and their extreme fragility prevent detailed ecological studies of Rhizaria. Indeed, rhizarians belonging to groups such as the collodarians, phaeodarians or acantharians are easily destroyed upon collection with standard sampling procedures (e.g. plankton nets; Dennett *et al.* 2002; Remsen *et al.* 2004) or dissolved when using common fixative (e.g. formaldehyde; Beers & Stewart 1970).

Therefore, very little information are available to estimate rhizarian biodiversity and how it is affected by environmental and temporal variability.

In the last decades, the development of high-throughput methods have proved to be a powerful tool to explore the environmental diversity of protistan communities and this approach regularly highlighted the high diversity and contribution of some rhizarians taxa (e.g. Collodaria) in marine ecosystems (Not *et al.* 2007; Sauvadet *et al.* 2010; Edgcomb *et al.* 2011; Pernice *et al.* 2015; Biard *et al.* in prep). Although they provided extremely valuable insights on plankton diversity and ecology, metabarcoding surveys over large spatial scales often ignored the seasonality of plankton communities, thus leading to potential erroneous interpretation of their ecology (Nolte *et al.* 2010). However, more attention has been recently brought to explore the effects of temporal variability of environmental parameters on plankton communities (e.g. temperature, nutrient limitation, etc.; Kim *et al.* 2014; Egge *et al.* 2015).

Here we monitored the seasonal dynamics of the plankton community in the Bay of Villefranche-sur-Mer during a period of 18 months using high-throughput methods. We assessed more specifically the contribution of the different rhizarian taxa to the global plankton community and determined the importance of different environmental variables driving the temporal variability of their taxonomical composition.

MATERIAL AND METHODS

Sample collection

Samples were collected monthly at Point B in the Bay of Villefranche-sur-Mer (France, 43°41’10’’ N, 7°18’50’’ E) between February 2013 and June 2014. At each date, 12 L of seawater were collected at 5 different depths (2, 15, 30, 45 and 60 m) using Niskin bottles and all 5 samples were mixed together to provide an integrated water sample over the full depth. Seawater samples were then pre-filtered through a 50 µm sieve to remove large planktonic organisms and 5L were thereafter filtered on 0.8 µm polycarbonate membranes. In parallel, plankton samples were also collected by vertical net tows (50 µm mesh size). After homogenisation of the cod-end content, 500 ml were filtered on 8 µm polycarbonate membranes. All filters were transferred into cryotubes, flash-frozen in liquid nitrogen for 15 min and stored at -80°C until DNA extraction.

Bi-monthly plankton samples were collected by 0-75 m net tows (50 and 200 µm mesh size nets). The 200 µm samples were immediately preserved in formalin buffered with borax (sodium borate) until saturation. The 50 µm plankton samples were first filtered through a 200 µm mesh size filter to remove large organisms and finally preserved in 2% lugol.

Environmental parameters

Environmental variables were provided by the long-term hydrological time-series, operated as part of the Service d’Observation en Milieu Littoral (SOMLIT/CNRS-INSU). A subset of 16 environmental variables was sampled on a weekly basis at 6 different depths: 0, 10, 20, 30, 50 and 75 m. Further information on environmental sampling methods is available at the

SOMLIT website (http://somlit.epoc.u-bordeaux1.fr/fr/). For each sampling date, each variable was averaged over the full depth.

Digital imaging acquisition

The preserved plankton samples, collected with the 200 µm mesh size net, were sorted using the ZooScan digital imaging system (Gorsky *et al.* 2010) as previously described (Vandromme *et al.* 2011). For the lugol fixed samples, we used the FlowCAM digital imaging system (Sieracki *et al.* 1998).

DNA extraction and Illumina sequencing

Genomic DNA was extracted using the NucleoSpin Plant II kit (Macherey Nagel, Hœrdt, France) following the manufacturers instructions. Extracts were quantified using a NanoDrop spectrophotometer (Thermo Scientific). We used the universal primers set TAReuk454FWD1 and TAReukREV3 (Stoeck *et al.* 2010) to amplify the V4 regions (ca. 380 bp) of the 18S rRNA gene. All PCR amplifications were conducted in a PCR workstation as previously described (Massana *et al.* 2015). Amplified products were checked on a 1% agarose gel. Each sample was purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel). Purified PCR products were quantified using Qubit Fluorometer (Fischer Scientific, Illkirch, France) and pooled amplicons were sent for sequencing using Illumina MiSeq at FASTERIS (Geneva, Switzerland).

Analyses of Illumina sequences

A total of 30 samples were retrieved after the sequencing step. Preliminary quality control analysis was performed by using the FASTQC software v0.10.0 (http://www.bioinformatics.-babraham.ac.uk/projects/fastqc/). We removed reads smaller than 150 bp and filtered the trimmed reads using the fastq quality filter (http://hannonlab.cshl.edu/fastx_toolkit/). Reads where at least 75% of the bases had a Phred score <20 were removed. Quality-checked trimmed reads were analysed in Mothur (Schloss *et al.* 2009) and further clustered into Operational Taxonomic Units (OTUs) with a 97% similarity threshold after removing sequences with less than 10 reads. We applied a chimera detection using UCHIME (Edgar *et al.* 2011) with default parameters. Taxonomy assignment was finally conducted by comparing the OTUs with a reference database derived from the Protist Ribosomal Reference (PR2) database (Guillou *et al.* 2013).

Data analyses

All data analyses, statistics and interpolations presented below were performed using R 3.2.0. (R Core Team 2015) and the ggplot2 (Wickham 2009), vegan 2.3-0 (Oksanen *et al.* 2015), akima 0.5-11 (Akima *et al.* 2013) packages as well as custom scripts. We performed non-metric multidimensional scaling (NMDS) of Jaccard distances between the sampling dates, to examine the similarity in OTU composition between samples by transforming the data to presence-absence prior to the analyses.

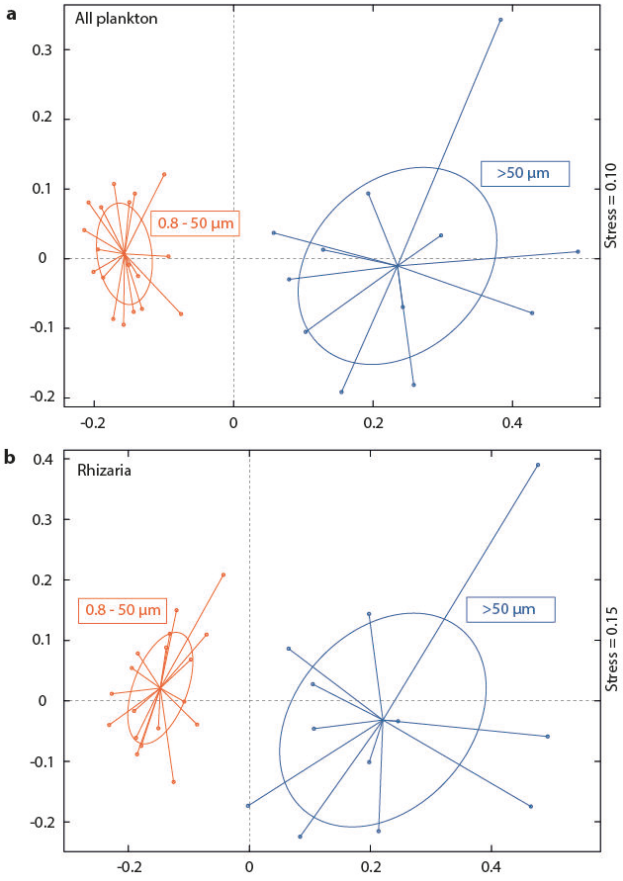

Figure 1 Non-metric multidimensional (NMDS) ordination of plankton community obtained from the 0.8 – 50 µm fraction (orange) and the >50 µm fraction (blue), using presence-absence transformed Jaccard similarities between all sampling dates. (**a**) NMDS ordination of the global plankton community. (**b**) NMDS ordination of the rhizarian community only.

PRELIMINARY RESULTS

Diversity of the plankton community

The diversity of the plankton community analysed by high-throughput sequencing included 6 253 488 sequences, clustered at 97% similarity in 3 626 OTUs. Analysis of both size fractions collected revealed that the small size-fraction (0.8-50µm) was twice more diverse (3 341 OTUs) than the larger one (>50 µm, 1 194 OTUs). In addition to the difference in diversity content, the fractions showed a clear difference in OTU composition as seen in the non-metric multidimensional scaling analysis based on OTU occurrence (Figure 1a). Following OTU taxonomic assignation, we found that Alveolates dominated the contribution to the smallest size fraction community throughout the period sampled (47% of sequences), followed by the Stramenopiles (17%), (Figure 2a) while the contribution of the larger size-fraction community was largely dominated by the Opisthokont (92%) followed by the Alveolates (4%; Figure 2b). Overall, Rhizaria were rather rare across samples, with low relative contributions to plankton communities in both size-fractions (3% and 1%).

Focusing on the Rhizaria, they appeared more diverse in the small size-fraction (513 OTUs) than the large size-fraction (119 OTUs). The two fractions were also clearly differentiated based on the OTU occurrences (Figure 1b). While the Cercozoa phylum (here represented by the Thecofilosea, the Imbricatea and the Chlorarachnea) contribute up to half of the rhizarian sequence abundance in the 0.8 – 50 µm fraction, acantharian and radiolarians belonging to the "RAD-B" group, had medium contribution (Figure 2c). The less diverse, larger size fraction (>50 µm), was clearly dominated by the Phaeodaria (73%), with the exception of two samples, where the Filosa lineages contributed most (Figure 2d).

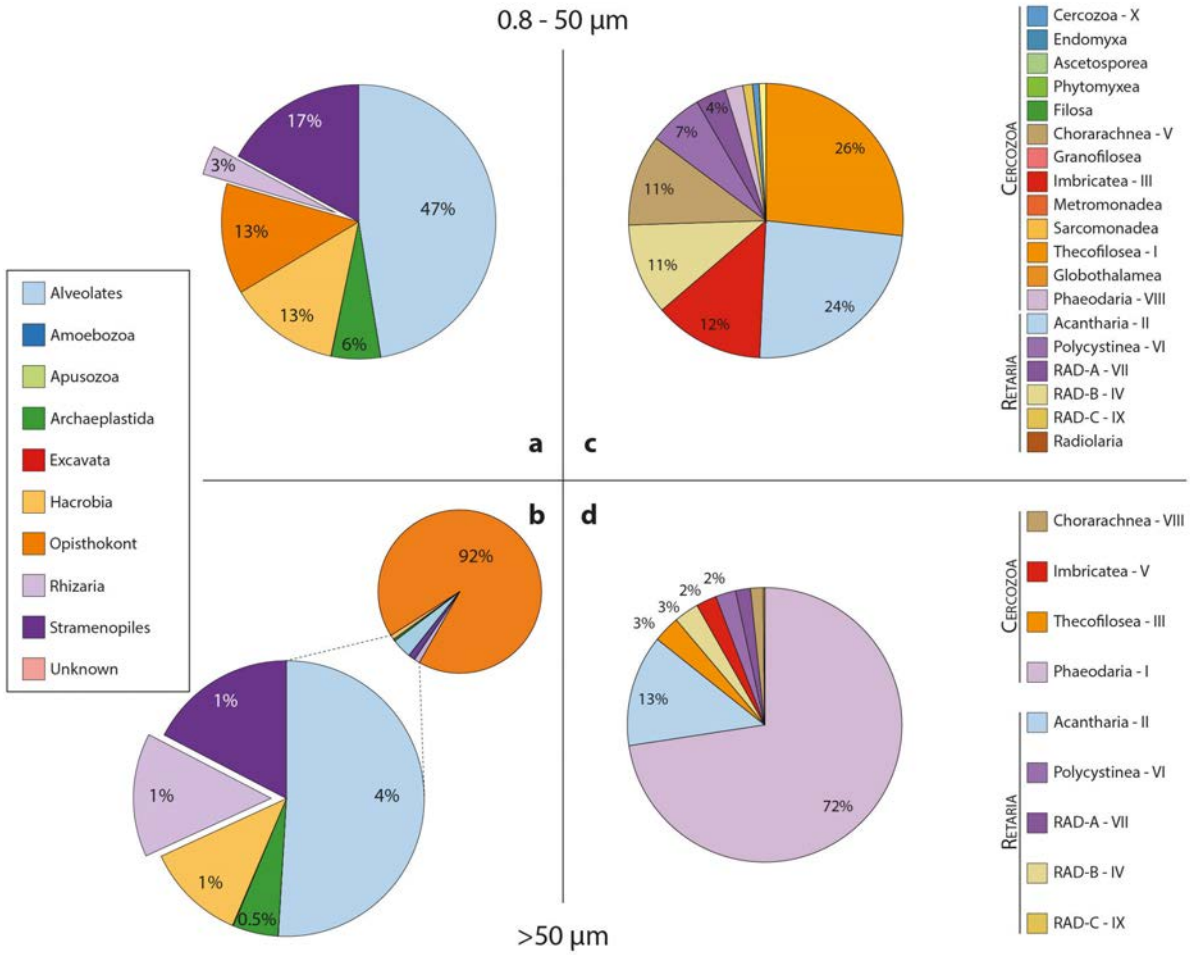

Figure 2 Diversity of 18S rDNA sequences relative abundances assigned to high-rank taxa. Pie charts display the average contribution of high-rank taxa to the global community and the Rhizaria community The rank of the ten most abundant rhizarian taxa is highlighted on the right legends. (**a**) Fraction 0.8 – 50 µm. (**b**) Total diversity of the fraction larger than 50 µm. (**c**) Rhizarian diversity in the 0.8 – 50 µm fraction and (**d**) the rhizarian diversity in the fraction larger than 50 µm.

Seasonality of plankton diversity

The seasonality of plankton community observed at our sampling point, revealed a strong temporal dynamic, with winter and spring samples being separated from the summer and fall samples along the first NMDS axis (Figure 3a). Such seasonality appeared clearer in the small size-fraction than in the larger (Figure 3b), although the later had less sampling dates. The OTU composition showed that the seasonality observed in the 0.8 – 50 µm fraction, was mainly driven by the Alveolates, though some Stramenopiles (e.g. diatoms) had much greater contribution during March (Figure 4a). When excluding the Opisthonkonts, the Alveolates, Hacrobia and Stramenopiles were also dominant throughout spring until the early summer (Figure 4c). During the late summer and fall, the Rhizaria increased their contribution to the plankton community in the Bay.

The seasonality of the rhizarian community in the small size-fraction showed similar patterns to the global plankton community, as revealed by the Jaccard similarity between sampling dates in the NMDS (Figure 5a). The results confirmed the observation made for the

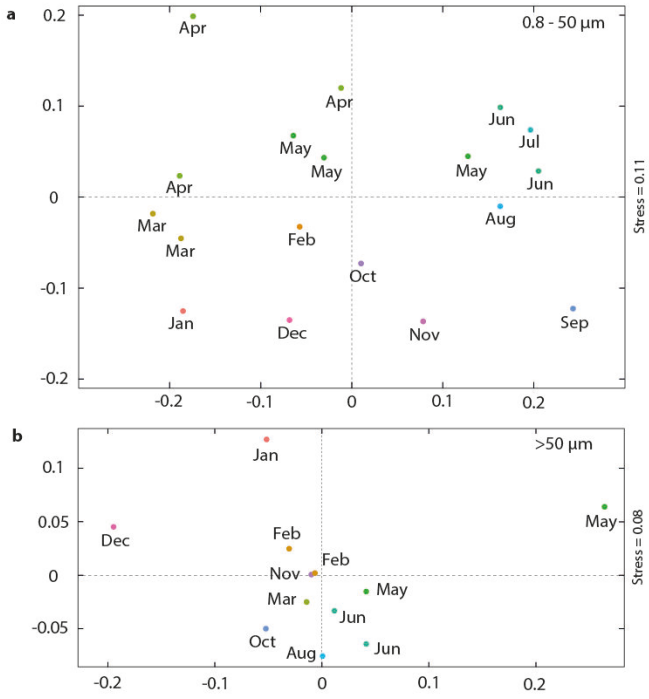

Figure 3 Seasonality of the plankton community. Non-metric multidimensional (NMDS) ordination of plankton community using presence-absence transformed Jaccard similarities between all sampling dates. (**a**) NMDS ordination of the 0.8 – 50 µm fraction. (**b**) NMDS ordination of the >50 µm fraction.

global community, suggesting a shift in OTU occurrence between samples from the period ranging from the early fall to early spring (ca. October to March), and a period including the spring and the summer (ca. April to September). In colder and deeply stratified water conditions (Figure 6), we observed an increasing contribution of the RAD-B group, as well as other groups like the phaeodarians or the RAD-A group (Figure 7a). In the warming and stratified waters of spring, the Cercozoa displayed increase contribution to the rhizarian community (Figure 7a). During this period this diverse phylum, including the Thecofilosea or the Imbricatea, had the highest contributions, the later having increasing contribution in the early spring. During this same period, the sample from June 2013 was clearly difference from the general trend, with a high contribution of Acantharia. We could not determine a clear temporality for the Rhizaria from the large size-fraction (Figure 7b), although several months (i.e. February 2014, March 2014 and May 2013-2014) clearly showed an important difference in OTU composition, with higher contributions of the Filosa and Acantharia (Figure 7b).

DISCUSSIONS AND PERSPECTIVES

Diversity of plankton community

Although a few attempts have been conducted to catalogue the plankton diversity in the Bay (Seguin 1981; Gómez & Gorsky 2003; Romagnan *et al.* 2015), most of this work was based on morphological taxonomical expertise on specific groups. The use of high-throughput sequencing (HTS) approach to assess the biodiversity of the plankton community in the Bay of Villefranche-sur-Mer gave some additional insights on poorly studied taxa. For instance, the diversity of Cercozoa revealed here, as well as their high contribution to the rhizarian community, suggest that these lineages, previously ignored in biodiversity investigation of the Bay, represent a highly diverse group that will surely deserve further examination to achieve a comprehensive understanding of the plankton ecology in the bay. Detailed contribution of the different rhizarian group also revealed that the group RAD-B was the fourth contributor to the rhizarian relative abundance in the 0.8 – 50 µm size-fraction (Figure 2c). This radiolarian group has been created recently (Not *et al.* 2007; Viprey *et al.* unpublished) to gather

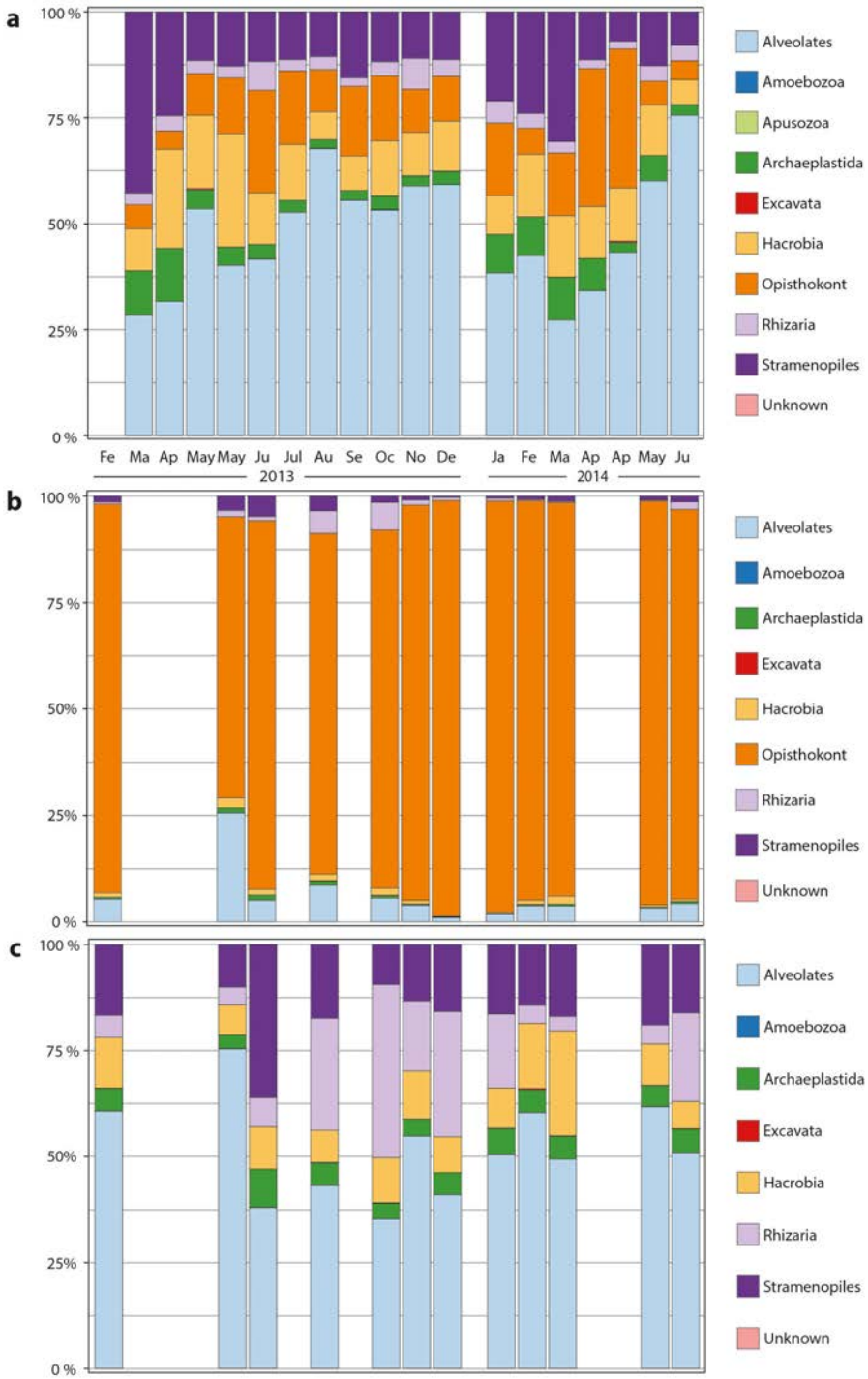

Figure 4 Seasonality of 18S rDNA sequences relative abundances assigned to high-rank taxa. (**a**) Seasonality of the 0.8 – 50 µm fraction. (**b**) The seasonality of the >50 µm fraction and (**c**) the detailed composition without the Opisthokont.

sequences similar the unique Taxopodia representative, the species *Sticholonche zanclea* (Suzuki & Not 2015). Although this group has been barely studied, the insights brought by environmental diversity surveys, suggest that this group is more diverse than the only species described. For instance, sequences belonging to the RAD-A and RAD-C groups, form two groups of Taxopodida radiolarian encompassing environmental sequences only (Suzuki & Not 2015). Beyond the specific study of rhizarian diversity, the use of HTS methods provided

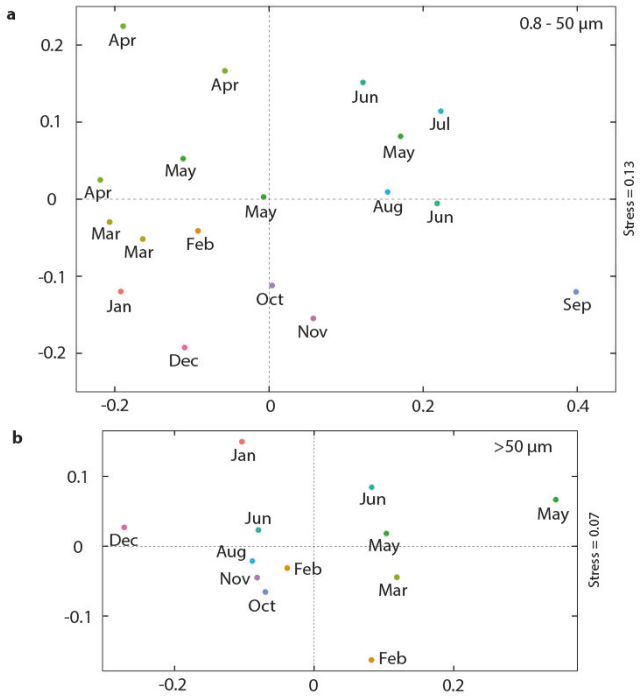

Figure 5 Seasonality of the rhizarian community. Non-metric multidimensional (NMDS) ordination of rhizarian community using presence-absence transformed Jaccard similarities between all sampling dates. (**a**) NMDS ordination of the 0.8 – 50 µm fraction. (**b**) NMDS ordination of the >50 µm fraction.

a large spectrum of plankton diversity (Figure 3) that will require special attention in order to compare the well-known diversity catalogues of the Villefranche-sur-Mer Bay, with the newly biodiversity explored through HTS methods.

Surprisingly, Collodaria were almost absent from any samples, including both size-fractions. Although they can be frequently encountered in the Bay, some times with high abundances (Biard, unpublished), the present sampling strategy failed to properly collect the Collodaria. This could be explained by: (i) the small volume collected with the Niskin or, (ii) the size of the mesh used to collect plankton samples (50 µm), which is not designed to collect the large collodarian often sampled using larger mesh sizes (i.e. 300 µm). As they are typically distributed horizontally over the water-column, the vertical net-tows employed here, might have under sampled the Collodaria. Therefore, to better assess the contribution of Collodaria to the plankton community in the Bay, the sampling strategy should be revaluated to allow the collection of larger planktonic components. This could be carried using Regent Nets (680 µm) by oblique or horizontal net tows.

Temporal variability of specific plankton taxa

The presence of a plankton time-series since 1966 allowed general studies of plankton seasonal dynamics (Gómez & Gorsky 2003; Vandromme *et al.* 2011; Romagnan *et al.* 2015), but also detailed studies of specific taxa such as copepods (Seguin 1981) or pteropods (Howes *et al.* 2015). This current analysis of the plankton seasonality, with a focus on Rhizaria, is therefore included in a well-documented ecosystem allowing multiple comparisons with previous works.

In the present preliminary results, the seasonality was explored at two different taxonomical levels, the super-group and considering only the different lineages included in the Rhizaria (Figures 3-5, 7). Since these levels are far from being resolutive enough to answer detailed ecological questions, more complete investigation should be carried to better assess the importance of specific taxa into the seasonality observed here. The contribution of autotrophic taxa such as the blooming diatoms or the photosynthetic rhizarian (i.e. Chlorarachnea and Imbricatea; Lauterborn 1895; McFadden *et al.* 1994) should be explored.

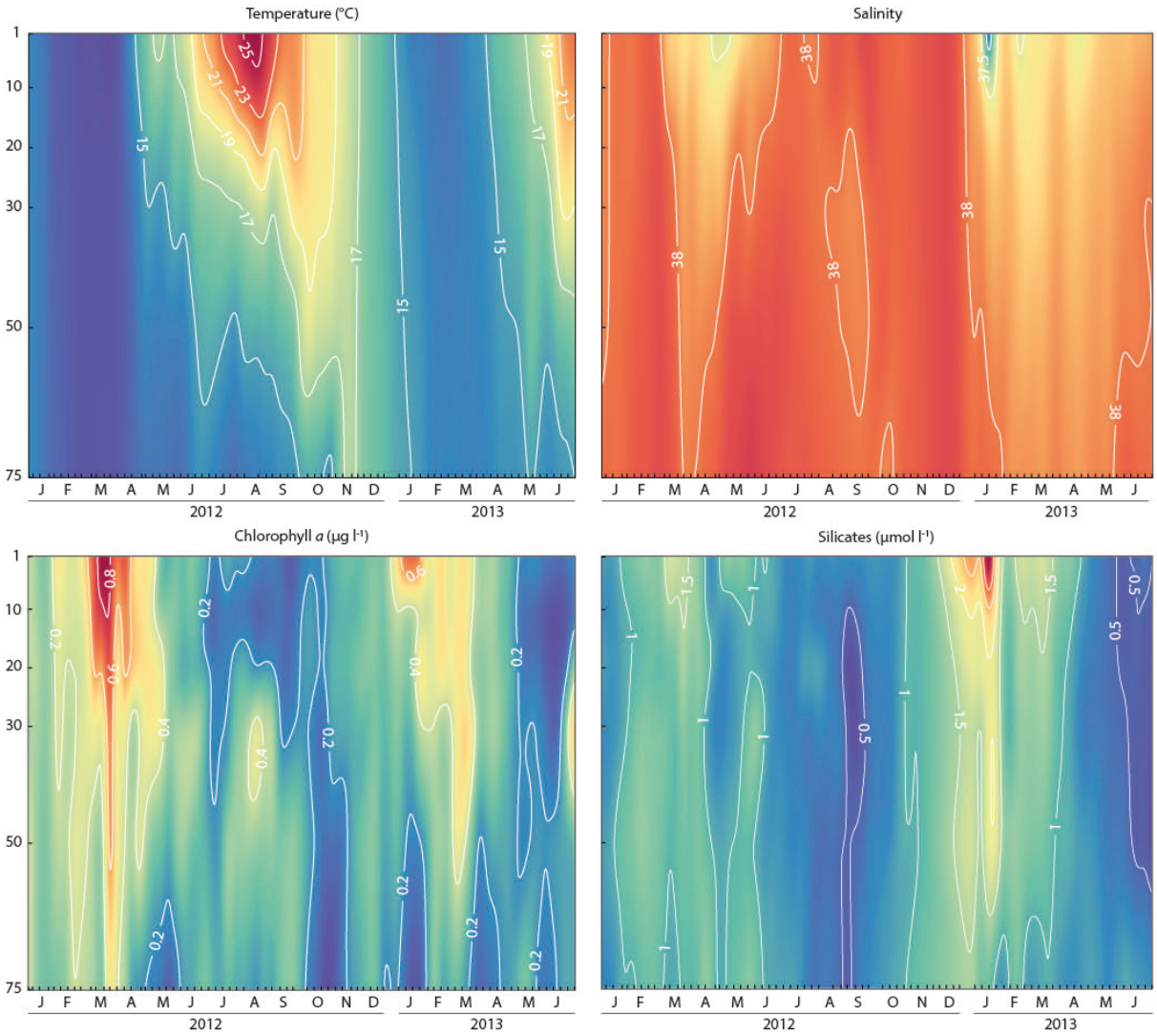

Figure 6 Environmental conditions at point B. Data were sampled weekly at five depths, from January 2013 to July 2014. Isolines are displayed in white for each of the variables.

As we see it now, the contribution of Stramenopiles seems more important in early spring, during the period where spring bloom often occur in the Bay (Gómez & Gorsky 2003). Although these blooming condition are often mainly investigated through the well-known groups like diatoms, it would interesting to better assess the contribution of the poorly sampled autotrophic Rhizaria.

Within the different rhizarian lineages, the radiolarian group RAD-B showed a clear temporal variability, contributing more between October and February. The seasonal pattern observed in the Bay of Villefranche-sur-Mer, suggesting an increase in fall, is not consistent with the few other observations of Taxopodida, previously describing them as being more abundant between spring and fall (Tan *et al.* 1978; Klaas 2001). However, further analyses should be performed to better assess the effects of environmental variables on the specific temporal dynamics of Taxopodida in the Bay of Villefranche-sur-Mer.

Additionally, Phaeodaria were the dominant rhizarian group along time in the >50 µm size fraction (Figure 7). This important group might actually correspond to the genus *Aulacantha*, a well-known Phaeodaria in the Bay and a dominant component of the Rhizaria

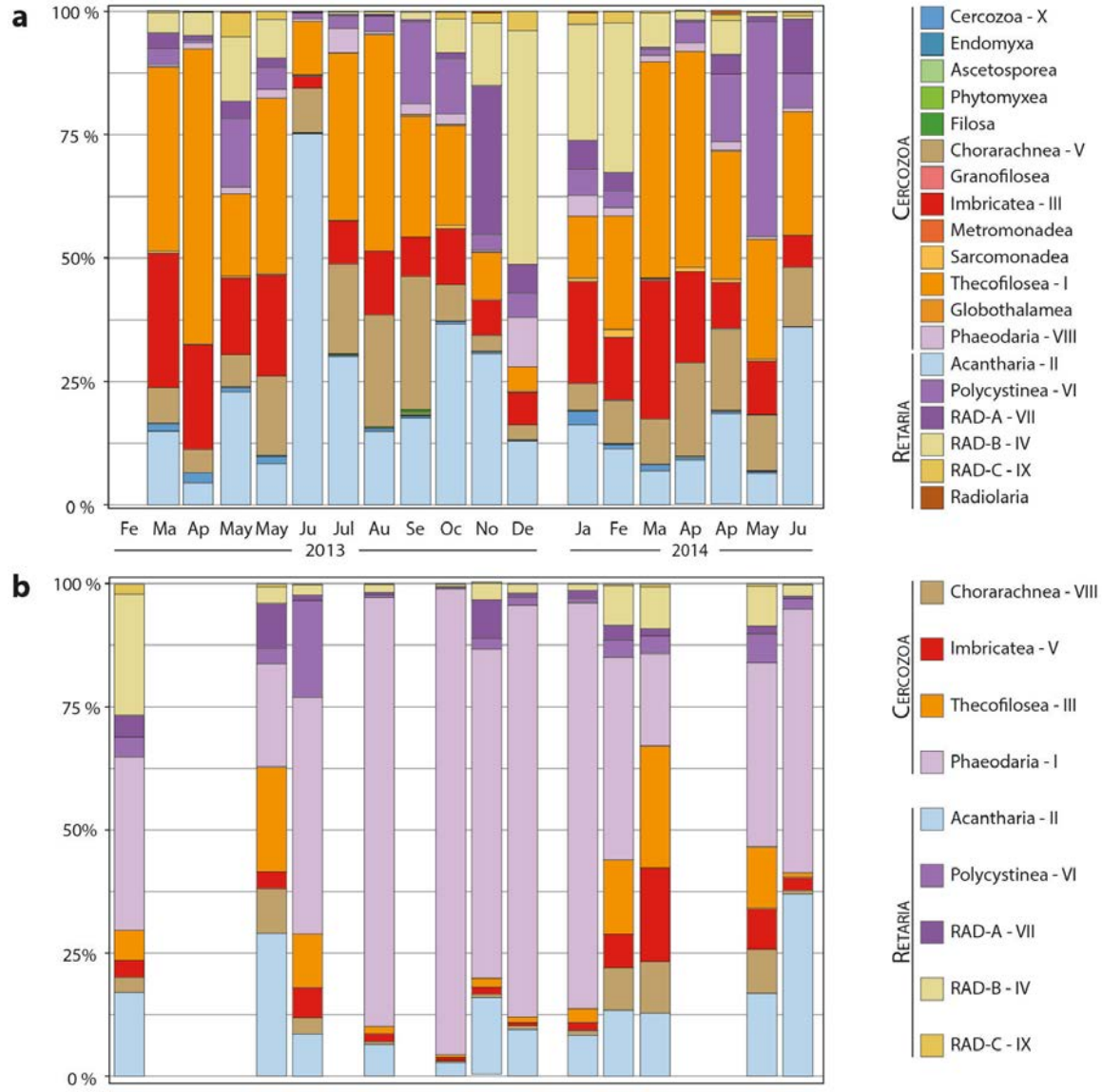

Figure 7 Seasonality of 18S rDNA sequences relative abundances assigned to the Rhizaria. The rank of the ten most abundant taxa is highlighted on the legend. (**a**) Seasonality of the 0.8 – 50 µm fraction. (**b**) The seasonality of the >50 µm fraction.

as seen by the ZooScan (http://www.obs-vlfr.fr/data/files/~ccz/QueryCCZ-PTB.php). In the context of our time series study, the simultaneous bi-monthly collection of plankton samples dedicated to ZooScan and FlowCAM analyses, will allow such direct comparison between HTS diversity results and the quantification of plankton diversity and abundances assessed by digital imaging systems.

Ecological successions

Ecological successions of the plankton community from the Bay of Villefranche-sur-Mer have been already investigated using standard analysis procedures (e.g. microscope, ZooScan, cytometry, etc.; Romagnan *et al.* 2015). However such approaches have a limited taxonomical resolution for rare and fragile taxa, in particular the Rhizaria. Using high-throughput

sequencing (HTS) procedure, we provide an alternative, potentially more comprehensive dataset to investigate the ecological successions within the plankton community. As ecological succession of plankton communities are strongly influenced by physical processes such as mixing or light availability (Levasseur *et al.* 1984), the weekly sampling of environmental variables in the Bay will allow the direct comparison between plankton diversity dynamics and environmental variability. Using multivariate analyses (e.g. Principal Component Analyses) and variance partitioning approaches through Redundancy analyses (RDA), we will investigate and estimate the influence of specific environmental variables on diversity seasonality (e.g. Egge *et al.* 2015, Simon *et al.* 2015).

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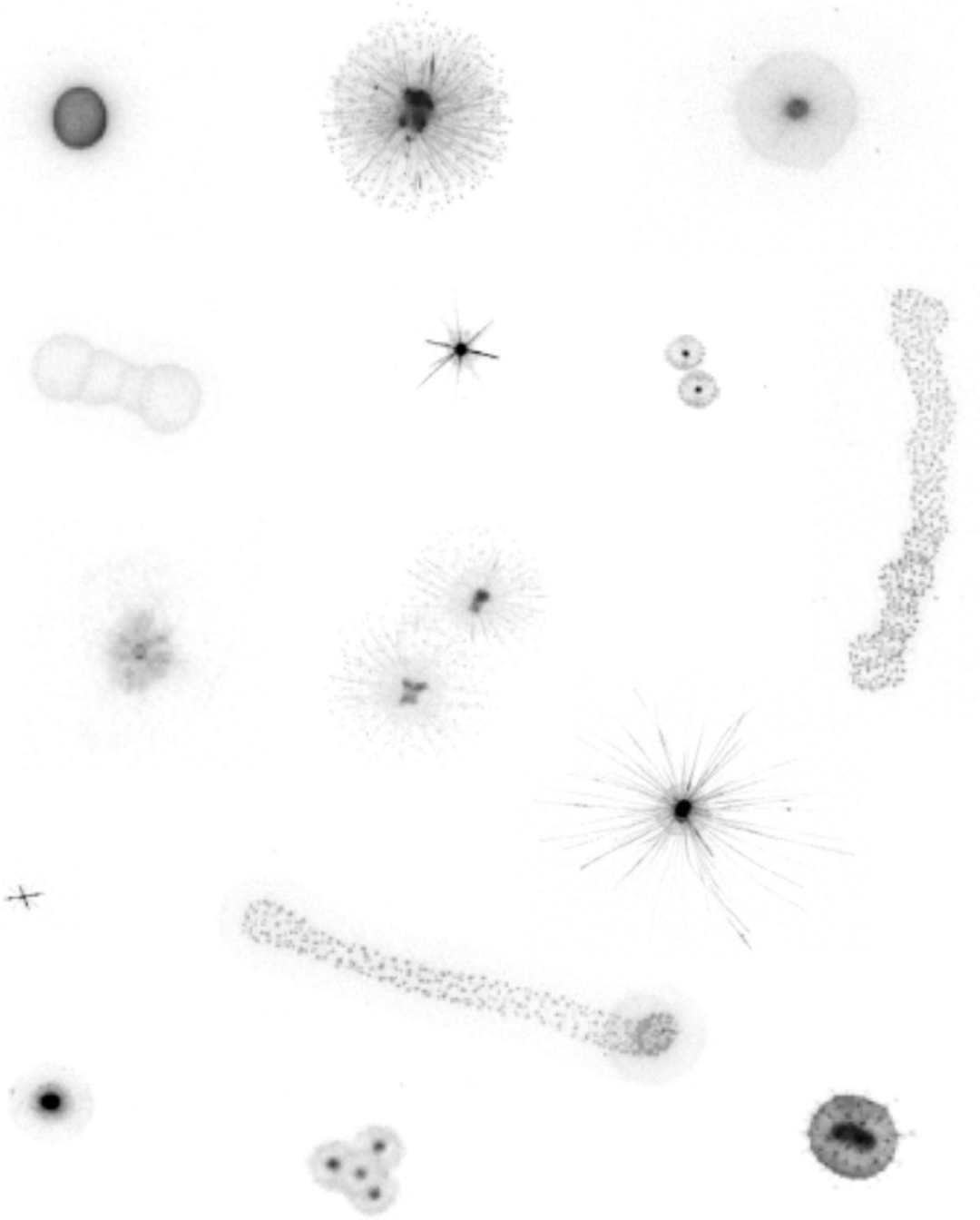

CHAPITRE III

RHIZARIA, THE ELUSIVE STARS OF THE OCEANS

« It is surrounded by the Radiolarian sea and the Ocean-bed at a depth of three English miles is covered with Radiolarian ooze. The Zephyrs of perpetual Summer blow from the Sea. The plants yeald nectar. The animals are as innocent as those of the Garden of Eden. The sands are pearls and the rocks are pure gold (…) »

John Murray to Ernst Haeckel (1900)

Global *in situ* imaging observations reveal the biomass of Rhizaria in the oceans.

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Abstract

Planktonic organisms play pivotal roles in oceanic food webs and global biogeochemical cycles[1,2]. Most of our knowledge on the ecological impact of large zooplankton stems from research on abundant and robust crustaceans, mainly copepods[3,4]. A number of other components of planktonic communities are more fragile, precluding efficient sampling with standard plankton net tows, meaning their abundances and impacts on the functioning of oceanic ecosystems are poorly known. Using data from a worldwide *in situ* imaging survey of plankton larger than 600 µm, we show here that the biomass of Rhizaria, a super-group of fragile unicellular marine protists including Radiolaria and Phaeodaria, represents an estimated standing stock of 0.075 petagrams of carbon (PgC) in the upper 200 m of world oceans, equivalent to 4.5% of the total oceanic biota carbon reservoir[5]. More specifically, in vast oligotrophic inter-tropical open oceans, rhizarian biomass is estimated to be equivalent to that of all meso-zooplankton, and photosymbiosis may be an important factor explaining their distribution. The previously overlooked importance of Rhizaria across the largest ecosystem on the planet[6] changes the perception of marine planktonic ecosystems, and is particularly relevant because climate simulations tend to predict that mid-latitude oligotrophic regions of the oceans will expand in the future[1,6].

Oceanic ecosystems are inhabited by a variety of planktonic organisms spanning a wide size range, from viruses to jellyfish. Plankton diversity and community structure are key drivers of oceanic ecosystem functioning and are centrally important for the ecology and biogeochemistry of the planet[1,2]. By feeding on small plankton, large zooplankton link primary production to higher trophic levels through the marine food web[7] and impact carbon export and remineralization to deep oceans by producing fast sinking particles (fecal pellets and dead bodies)[8]. Most of our knowledge about the ecological impact of these organisms stems from research on crustacean copepods, euphausiids and decapods[3,4] that are abundant, robust and easy to collect with standard methods such as plankton net tows. As a result, the zooplankton compartment in ecosystem and biogeochemical models is often exclusively represented by the physiological characteristics of copepods[9].

In contrast, the biology and ecology of planktonic Rhizaria, one of the main eukaryotic super-kingdoms, is largely unexplored[10]. Rhizarians are unicellular organisms ranging in size from a few micrometers to several centimeters. They are predators, but some species also host obligate intracellular microalgal symbionts (i.e. photosymbionts)[11]. Most rhizarians produce mineral skeletons of calcium carbonate (Foraminifera), strontium sulfate (Acantharia), or silicate (Polycystinea, Phaeodaria). Rhizarian skeletons are often well preserved in marine sediments, making this group a primary focus for the development of paleoproxies[12]. A number of studies, ranging from sediment trap to environmental molecular surveys[13-15], provide hints as to the importance of Rhizaria in present day oceanic ecosystems. Recent

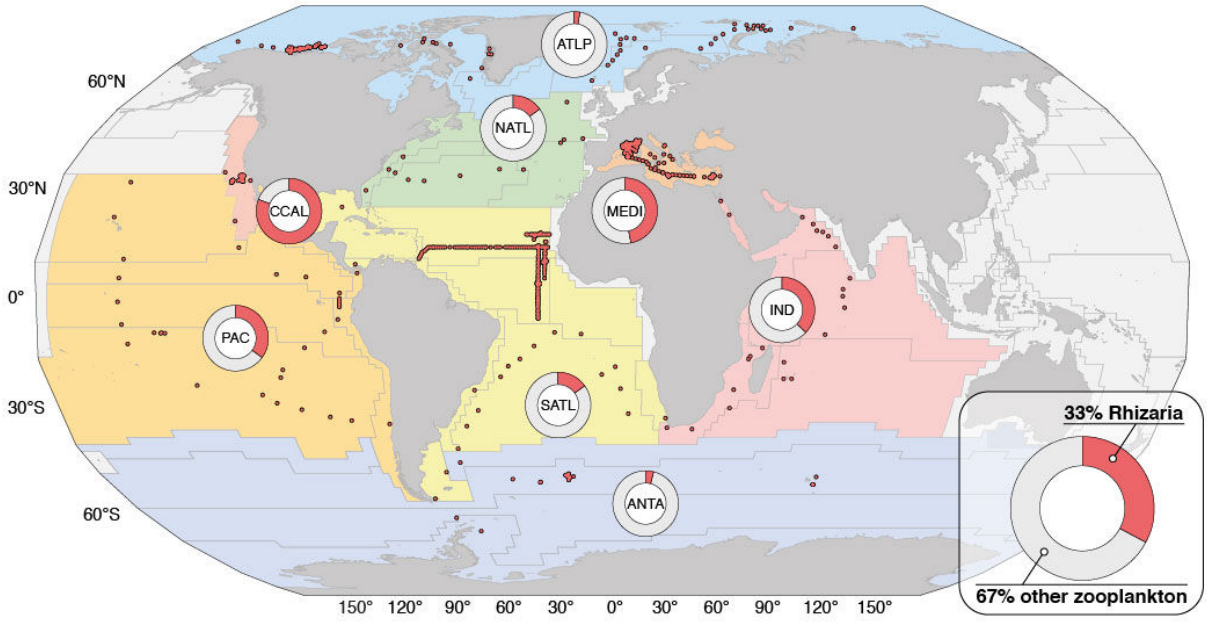

Figure 1 | Worldwide contribution of Rhizaria to zooplankton communities (>600 µm) in the upper 500 m of the water column. Underwater Vision Profiler sampling stations are represented by red dots (694 stations; Extended Data Table 1). Relative contributions of the depth-integrated abundances are shown for the Rhizaria (red) and other zooplankton (grey) as seen and quantified by the UVP5. The bottom right panel displays the global average contribution for each group considered. Contributions are geographically divided according to Longhurst's Biomes and Provinces[29] (numerical values are shown in Extended Data Table 2). Map made with Natural Earth data (http://www.naturalearthdata.com).

qualitative results from the *Tara* Oceans expedition demonstrated that the Collodaria, which are mainly large colonial rhizarians, are important components of plankton community structure[16,17] and are significantly correlated with downward fluxes of carbon[18]. However, as plankton nets severely damage these delicate organisms, they are inconsistently sampled[19,20] and their global distribution and role in the ecosystem are not well resolved. Previously observed as being abundant in specific areas of the oceans[19,21,22], their contribution to plankton communities has never been assessed on a global scale.

Using a non-destructive *in situ* imaging system (Underwater Vision Profiler - UVP5)[23], we quantified the respective contributions of Rhizaria and other zooplankton larger than 600 µm (i.e. meso- and macro-zooplankton, excluding smaller components of the plankton community) across a variety of pelagic ecosystems (Fig. 1, Extended Data Table 1 and Extended Data Fig. 1). Worldwide, in the upper 500 m of the water column, rhizarians comprised on average 33% of zooplankton observed (Fig. 1). Rhizaria were more abundant in the large inter-tropical oceanic basins, the Mediterranean Sea, and specific ecosystems such as the coastal upwelling off California (Extended Data Table 2). When converted to carbon biomass (Extended Data Table 3), the contribution of Rhizaria was highest between approximately 40°N and 20°S and was similar to the biomass of meso-zooplankton in the same latitudinal range[5] (Fig. 2). Overall, we estimate that at any given time the biomass of Rhizaria larger than 600 µm represents a standing stock of 0.075 Pg of C in the upper 200 m of the water column of the world ocean (Table 1). This biologically active carbon reservoir represents 26% of the combined meso- and macro-zooplankton biomass and 4.5% of the total oceanic biota carbon standing stock (Table 1), values that are consistent with previous data based on local studies[19] despite intrinsic uncertainties associated to such global estimates[5].

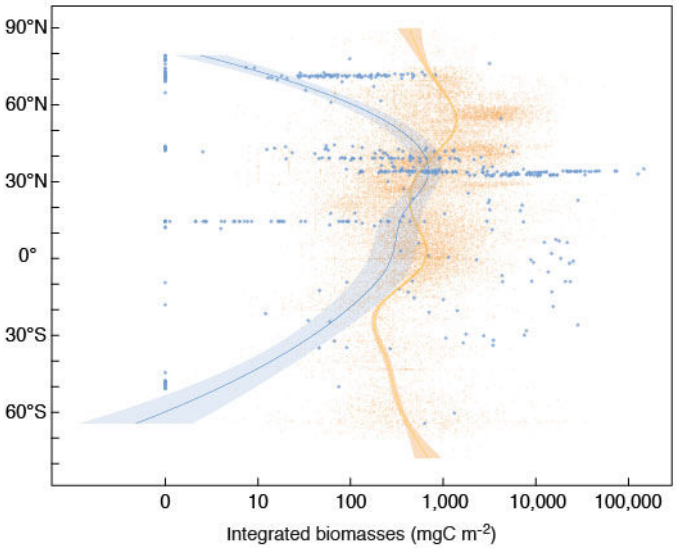

Figure 2 | Latitudinal distribution of depth integrated biomass (0-200 m depth) of Rhizaria (blue, *in situ* optical assessment, this study) and meso-zooplankton (orange, plankton net based assessments[30]). Loess regressions with polynomial fitting were computed to illustrate the latitudinal patterns. Pale color areas display a 0.95 confidence interval around the trends. Note that the biomass is plotted on a logarithmic scale.

The four main categories of Rhizaria discriminated in our analysis (Acantharia, Collodaria, Phaeodaria, and other Rhizaria; Extended Data Figs 2 and 3) exhibit distinct latitudinal biomass distributions (Extended Data Fig. 4). Overall, Phaeodaria and Collodaria were the most important contributors to rhizarian biomass, while Acantharia occurred at consistently low levels. The highest biomass of Collodaria and Acantharia occurred at low latitudes whereas Phaeodaria and other Rhizaria were more evenly distributed, suggesting distinct ecological preferences. In addition to latitudinal patterns, a significant shift in

taxonomic composition also occurred with depth (Fig. 3). In the upper 100 m of the water column, photosymbiotic Collodaria contributed most to rhizarian biomass (Fig. 3a). Below, in the twilight zones of the oceans (100-500 m depth), the asymbiotic phaeodarians were the most important contributors to rhizarian biomass at all latitudes (Fig. 3b).

Table 1 | Carbon standing stock of Rhizaria in the 0-100, 0-200 and 0-500 m depth layers of the oceans.

Depth layer (m)	Rhizarian integrated biomass ($mgC\ m^{-2}$)				Global rhizarian biomass (PgC)	Contribution to global:		
	Min	Max	Median	IQR		Carbon standing stock	Biomass of meso- and macro-zooplankton	Biomass of meso-zooplankton
0-100	0	23,910	34.43	247	0.012	—	—	—
0-200	0	146,400	207	1,341	0.075	4.5% (0.1-23%)	26% (1-69%)	27% (1-71%)
0-500	0	115,091	564	1,608	0.204	—	—	—

Estimates of global rhizarian carbon biomass were derived from median values and assuming an ocean surface of 3.61×10^{14} m^2. Rhizarian biomass contribution to global carbon and meso- and macro-zooplankton standing stocks were calculated based on median values for the 0-200 m depth layer (i.e. only matching data available globally) published in ref. 5. The ranges of contribution were computed using the first and third quartile of rhizarian integrated biomass, respectively. IQR = Interquartile range. Global biomass estimates are expressed in petagrams of carbon ($1\ Pg = 10^{15}$ g). Detailed computational processes are provided in the Methods section 'Global estimates'.

Considering that all Collodaria and most Acantharia investigated so far harbor symbiotic microalgae (photosymbionts)[22,24], we estimated that these groups typically contribute 0.18% (0.17% for Collodaria and 0.02% for Acantharia) of total primary production in oligotrophic waters (Extended Data Table 4). Very little primary data is available to estimate the contribution of Rhizaria to total primary production, yet it has been shown that, on occasion, large photosymbiotic Rhizaria can account for more than 1% of total primary production[25]. It should also be noted that our sampling was restricted to organisms >600 μm, therefore not covering the full size spectrum of acantharians, which have been estimated to contribute up to 20% of total primary production locally when considering their full size spectrum[24], and excluded the upper 5 m of the water column where collodarians can be highly abundant[22,25]. In this context, our estimates can be considered as conservative and further efforts are required to refine the emerging image of the global rhizarian contribution to primary productivity and biomass in the oceans. The previously overlooked but substantial contribution of rhizarian biomass to plankton communities changes our perception of the oligotrophic tropical oceans which represent one of the largest ecosystems on the planet, occupying nearly 40% of the Earth's surface[6], and are key biomes for the functioning of the biosphere. The use of appropriate tools provides new insights into global zooplankton community structure in the ocean, for instance demonstrating that photosymbiotic Rhizaria abundance declines less markedly than other, non photosymbiotic, zooplankton in oligotrophic environments (Extended Data Fig. 5), emphasizing the idea that photosymbiosis allows these large organisms to thrive in otherwise hostile environments[12,26]. To date, large rhizarians have been omitted from biogeochemical flux budgets but they may be efficient vectors for fluxes to the deep ocean *via* both primary production and vertical flux, therefore being key players in the biological pump[13]. Along with cryptic, fragile and transparent creatures like gelatinous plankton organisms, whose abundance likely remains poorly

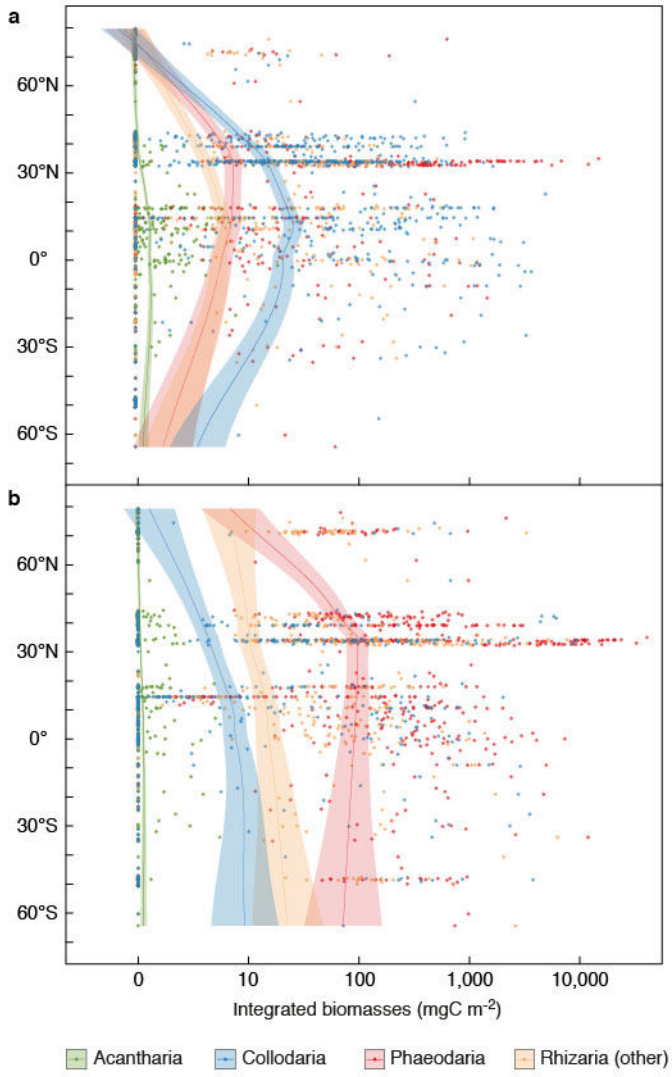

Figure 3 | Latitudinal distribution of depth integrated biomass (mgC m^{-2}) for the different rhizarian taxa identified. a, Biomass integrated in the upper 100 m of the water column (877 sampling stations). **b**, Biomass integrated between 100 and 500 m depth (694 sampling stations). Latitudinal trends are represented by computing Loess regressions with polynomial fitting. Pale color areas display a 0.95 confidence interval around the trends.

quantified[20], fragile rhizarians may thus contribute to carbon budgets in the dark mesopelagic oceans where the measured activity of microbial remineralization exceeds the estimated carbon input, emphasizing the need to understand the synergy between microbes and large zooplankton to understand processes controlling the oceanic carbon sink[27,28]. Beyond the global quantitative significance of Rhizaria, and along with better spatio-temporal description of the occurrence of specific taxa, accurate estimates of processes such as grazing, growth, biomineralization, photophysiology and carbon fixation of the symbionts are required in order to include this significant component of the oceanic biota in ecological and biogeochemical modeling at both local and global scales. This is particularly relevant in light of the fact that climate predictions show that mid-latitude oligotrophic oceans are likely to expand in the near future[1,6].

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Materials and Methods

Sampling sites. Rhizarian distribution was observed with the Underwater Vision Profiler 5 (UVP5) deployed at 877 stations distributed across all oceans (11 cruises corresponding to 1,454 profiles). Out of these stations, 694 were sampled down to 500 m depth (Fig. 1 and Extended Data Table 1). Stations encompassed regions with a broad range of oceanographic structures (upwelling, boundary currents, large tropical gyres, etc.) from oligotrophic to eutrophic ecosystems. Sampling effort occurred throughout the year between 2008 and 2013 and covered latitudes from 65°S to 75°N. The majority of stations (72%) were sampled within the 5°S to 40°N interval (Extended Data Fig. 1).

UVP5 deployments and raw data collection. The UVP5 images large plankton (Equivalent Spherical Diameter, ESD >600 μm; ref. 23). The UVP5 sampling volume varied from 0.5 to 1 L and images were recorded every 5 to 20 cm along vertical profiles, leading to an observed volume of 5 m^3 for a 500 m depth profile. Mounted on a CTD rosette frame, the UVP5 starts recording below 5 meters, ultimately leading to an underestimation in the quantification of objects just beneath the sea surface. Images produced by the UVP5 were extracted using the ZooProcess software[31]. For all objects, the major and minor axes of the best fitting ellipses were measured. A computer-assisted method was used to classify all organisms. Image identification was possible for objects larger than 600 μm. All images (total number ~1.8 million) were checked by experts to discriminate Rhizaria (~36,000 images) from other plankton and detritus. In the present dataset, the maximum ESD recorded was 7 cm (for a ctenophore) while it was 2.5 cm (for a colonial collodarian) when considering the Rhizaria only. Thereafter, Rhizaria were classified into finer taxonomic levels for all profiles included in this work. Extracted images data including all parameters can be downloaded at PANGAEA (XXX).

Refining the rhizarian images categories. The entire UVP5 image collection was scanned to infer the diversity of images associated to the Rhizaria. Based on taxonomic expertise, 10 categories were created and affiliated to known rhizarian taxa (Extended Data Fig. 2). Differences in shapes and grey level were used to help the distinction between categories. Phaeodaria were divided into 3 categories “PhaL”, “PhaSe” and “PhaSt”. The phaeodarians “PhaSe” (Extended Data Fig. 2a) are small grey spheres (<5 mm) with a tiny black nucleus inside. The black dot is mostly found in the centre of the sphere, however its position can vary. The edges of the sphere are darker than the interior (this being an important criterion to differentiate them from solitary collodarians). Such phaeodarians can be found in aggregates consisting of tens of specimens. Phaeodarians “PhaSt” (Extended Data Fig. 2b) are dark grey spheres with a large black or white nucleus. The interior of the sphere is entirely grey, unlike other phaeodarian categories. Tiny spines surround the sphere. Images of the phaeodarians “PhaL” (Extended Data Fig. 2c) are characterised by multiple long spine-like extensions originating from a dark centre. This dark centre can be a simple black dot or a grey sphere with a dark nucleus. Acantharians “Acn” (Extended Data Fig. 2e) possess short to medium size spines surrounding a black centre. The most important criterion to distinguish acantharians from phaeodarians “PhaL” in the UVP5 images is the symmetry of the spines characteristic of acantharian cells. Collodarians were divided into five categories including

colonial and solitary specimens. Large collodarian colonies “Col” (Extended Data Fig. 2j) are easily recognisable with their large size (often >5 mm) and their pigmented appearance. Colony shape is variable: spherical, stretched, assemblage of spheres, etc. A pale halo often surrounds the colony, this feature being helpful for identifying collodarian colonies. The solitary collodarians “SolGlob” (Extended Data Fig. 2i) are large spherical to oval organisms with a homogenous grey (or dark-grey) surface. The central sphere is surrounded by a blurry halo generated by a network of pseudopodial extensions. The solitary collodarians “SolB” and “SolG” (Extended Data Fig. 2f, h) are large spherical organisms with a dense central part (respectively black/dark grey and grey). A gradient of grey is observed from the central part to the outer part of the cell. As the outer most part of this halo is almost transparent, the edges of the organism will not always be seen. The grey level of the central part is the main criterion to distinguish the two categories. The last category of solitary collodarians “SolF” (Extended Data Fig. 2g) also comprises large spherical organisms with a grey central part surrounded by a dark-grey fuzzy structure. A gradient of grey is observed from the central part to the outer part of the cell. The fuzzy structure around the central part is the main criterion to distinguish these organisms from other “solitary” categories. Not all rhizarian images fitted into the criteria defined above to distinguish the different categories. The category “Rhiz” (Extended Data Fig. 2d) contains rhizarians that cannot be precisely classified in the previous categories.

Qualitative calibration of the newly defined categories was performed on plankton samples gently collected in Villefranche-sur-Mer bay (France, 43°41’10’’N, 7°19’00’’E) using a Regent net (680 µm mesh size) and off California (Californian Current Ecosystem) using a 333 µm mesh size plankton net hauled at a maximum speed of 0.5 m s^{-1} to minimize damage to the specimens. Live rhizarian specimens (Collodaria and Phaeodaria) were handpicked from the samples and then transferred into 0.2 µm filtered seawater. Each specimen was identified and photographed. The UVP5 was immersed in an aquarium filled with 0.2 µm filtered seawater. Freshly isolated specimens were dropped one by one on top of the illuminated volume of water to capture *in situ* images. Comparison between *ex situ* and *in situ* images confirmed the categories defined for the UVP5 image collection (Extended Data Fig. 3).

Data analysis. Analyses of rhizarian data included five steps: (i) vertical binning of each profile in four depth layers (0-100 m, 0-200 m, 100-500 m and 0-500 m). These layers were selected based on photic properties, availability of published matching plankton datasets for comparisons and to maximize the number of sampling stations considered in our dataset. Then for each depth layer we calculated (ii) the integrated abundance and (iii) biomass of all Rhizaria, and (iv) the primary production by photosymbiotic Rhizaria. (v) Results were averaged according to the different biogeochemical regions. All analyses were performed in *R*[32] with the package ggplot2 (ref. 33).

(i) For each sampling station with several vertical profiles, all profiles were summed to construct one single profile. Stations were divided into two categories based on the maximum deployment depth. The UVP5 recorded images down to 100 m depth in 877 stations, and down to 500 m depth in 694 stations (Extended Data Table 1). Sample volume was on average 1.74 ± 0.59 m^3 between 0 and 100 m depth and 2.95 ± 0.81 m^3 between 100 and 500 m depth.

(ii) Mean integrated abundances and relative contributions of Rhizaria were calculated for the 0-500 m layer. To assess the contribution of Rhizaria to the entire zooplankton community, the abundance of other planktonic groups, identified during the image process, was computed. All other zooplankton imaged by the UVP5 have been discriminated from non-living particles by semi-automatic annotation validated by experts and included copepods, crustaceans (shrimp-like, amphipod, cladoceran), gelatinous zooplankton (jellyfishes, ctenophores, siphonophores, salps), chaetognaths, appendicularia, molluscs, annelids and fish larvae. Other particles (detritus, aggregates, etc.) and phytoplankton (large diatoms, *Trichodesmium*, etc.) were removed from the computation.

(iii) Biomass estimations for the different rhizarian categories were inferred from organism measurements (major and minor axes of the best fitting ellipse) generated during the ZooProcess image processing. These axes were used for biomass calculation instead of the Equivalent Spherical Diameter (ESD), as the use of the latter leads to an overestimation of biomass for large and elongated organisms (such as long colonial collodarians). Biovolume was first calculated from geometric shapes for all categories except colonial collodarians: spheres for Acantharia, prolate ellipsoid for all other categories (Extended Data Table 3). Areas (A_e) of a prolate ellipsoid were determined for colonial collodarians as follows:

$$A_e = 2\pi \cdot \left(\frac{minor}{2}\right)^2 + \frac{2\pi \cdot \left(\frac{Major}{2} \cdot \frac{minor}{2}\right)}{e} \cdot \arcsin(e) \quad (1)$$

where e is the eccentricity of an ellipse:

$$e = \frac{\sqrt{\left(\frac{Major}{2}\right)^2 - \left(\frac{minor}{2}\right)^2}}{\left(\frac{Major}{2}\right)} \quad (2)$$

Biovolumes and surface areas were then converted to biomass using carbon conversion factors from the literature (Extended Data Table 3).

(iv) Primary production of photosymbiotic rhizarians was estimated individually for all Acantharia and Collodaria observed between 0 and 500 m. While all collodarian species investigated have been described as photosymbiotic[22], the vast majority of large acantharian specimens found in the upper water column is known to harbour symbionts[24,25]. Individual primary production (*iPP*) was estimated for each photosymbiotic rhizarian as a function of the biovolume[25]:

$$\log(iPP) = 0.62 \cdot \log(Biov) - 4.33 \quad (3)$$

where *Biov* is the biovolume of the holobiont estimated from a prolate ellipsoid.
Assuming a reference temperature (T_{ref}) of 23.5 °C (ref. 25), we applied a Q_{10} temperature coefficient of 1.88 (ref. 34) to correct temperature effects on photosynthetic production and get to temperature corrected individual primary production (iPP_T):

$$iPP_T = iPP \cdot \left[1.88^{\left(\frac{T_{ref} - T_{ctd}}{10}\right)}\right]^{-1} \quad (4)$$

where T_{ctd} is the *in situ* temperature measured by the CTD at the depth of each organism.

The 490 nm light attenuation coefficient $K_d(490)$ (m^{-1}) and photosynthetic active radiation PAR (mol photons m^{-2} day^{-1}) data were used to estimate the available instantaneous radiation at depth. Satellite derived average daily PAR, net primary production (NPP), chlorophyll *a* (Chla_{sat}) and the $K_d(490)$ data (8 day averages at 4 km resolution) were downloaded from the Oregon University database (http://www.science.oregonstate.edu/ocean.productivity/). Average values for each station were calculated for the position occupied ± 0.1° if the occupation date fell within the 8 day window of the satellite observation. The PAR attenuation coefficient K_d(PAR) was calculated according to Morel[35] as:

$$K_d(\mathrm{PAR}) = 0.0864 + 0.884\,K_d(490) - 0.00137\,[K_d(490)]^{-1} \quad (5)$$

The average instantaneous radiation available at the surface ($iPAR_s$ in µmol photons m^{-2} s^{-1}) during daytime was calculated as:

$$iPAR_s = \left(\frac{PAR}{daylength}\right) / 3600 \cdot 10^6 \quad (6)$$

with daylength in hours per day. From this data, the instantaneous radiation available at a specific depth z ($iPAR_d$) was calculated as:

$$iPAR_d = e^{(K_d(\mathrm{PAR}) \cdot z)} \cdot iPAR_s \quad (7)$$

Primary productivity measurements for radiolarians and acantharians used in Equation (3) were conducted at surface light conditions nearby Bermuda[25]. These conditions are likely saturating light conditions for symbiotic rhizarians[36]. Very little photo-physiological information is available for photosymbiotic Rhizaria. To calculate the decrease in primary productivity with decreasing light availability, the fraction of primary productivity possible at a given *iPAR* (fPP_{iPAR}) was calculated from the light saturation intensity (I_k = 165 µmol photons m^{-2} s^{-1}) observed for *Globigerinoides sacculifer*, a photosymbiotic planktonic Foraminifera[36] according to the hyperbolic tangent function for the light dependency of photosynthesis in marine phytoplankton[37] as:

$$fPP_{iPAR} = \tanh\left(\frac{iPAR_d}{I_k}\right) \quad (8)$$

The individual primary productivity at a given depth was then calculated as:

$$iPP = iPP_T \cdot fPP_{iPAR} \quad (9)$$

Finally, given the paucity of information available, it should be noted that we used size – primary productivity relations for acantharians and collodarians from just one study[25] and the dependency of photosynthesis on light availability from a planktonic Foraminifera for these calculations.

(v) Each UVP5 station was finally affiliated to one of 33 Longhurst's Biogeochemical provinces and assembled in the biomes defined by Longhurst[29] (Extended Data Tables 1 and 2). Only 29 stations were sampled within Longhurst's coastal provinces (FKLD, BRAZ, CARM, CHIL, GUIA, NWCS, ARAB, REDS and EAFR). The bottom depth at these stations was always >500 m and they were not located on the continental shelf or continental slope. All these coastal province stations were therefore merged with their adjacent oceanic biomes, while the Antarctic Biome included both the Antarctic Polar Biome and the Antarctic Westerly Winds Biome. The merging of sampling stations located in Longhurst's coastal

provinces with their adjacent biome did not impact the contribution of Rhizaria to zooplankton communities in these biomes. Indeed, when considered separately the average Rhizaria contribution for coastal provinces reaches similar values as for the global dataset (i.e. 33.81%). Two provinces, the Mediterranean Sea Province (MEDI) and the California Upwelling Coastal Province (CCAL) were treated separately from their respective biomes (Atlantic Coastal and Pacific Coastal Biomes) since both were densely sampled and showed high rhizarian abundances compared to the other provinces in the same biomes.

Global estimates. Global estimates of rhizarian biomass were computed for three different layers (Table 1) and assuming an ocean surface of 3.61×10^{14} m^2. All estimations were derived from median biomass values to prevent overestimations using mean values, the latter being highly influenced by locally high biomass values (e.g. California Current). Low and high estimations of global rhizarian biomass were computed using the first and third quartile, respectively. We compared the global rhizarian biomass to independent data on the global average estimates of meso- and macro-zooplankton biomass in the first 200 m of the oceans[5]. The contribution of Rhizaria to global plankton biomass was established using 11 different Plankton Functional Types (PFTs)[5] including autotrophic and heterotrophic PFTs. Median derived biomass for each plankton group was considered in the top 200 m and summed together to provide an estimation of the plankton carbon standing stock. Despite uncertainties inherent to any global estimates (e.g. carbon conversion factors, sampling coverage; ref. 5) we intended to provide a conservative contribution of Rhizaria to the different plankton components. The relative contribution of global rhizarian biomass to global plankton carbon standing stock was therefore calculated as the global rhizarian biomass, divided by the sum of the published reference estimate for global plankton biomass[5] and the global rhizarian biomass.

Possible impact of sampling coverage on rhizarian biomass distribution pattern. The global patterns observed in this study are inevitably associated to the sampling effort and geographic coverage (Extended Data Fig. 1). Some oceanic areas and/or seasons were more intensely sampled, creating heterogeneity in the dataset. For instance the Mediterranean Sea and the California Current were sampled intensely. Although we sampled 33 of 51 Longhurst's Provinces, our spatial coverage was partial. To assess the possible impact of sampling coverage on latitudinal pattern of rhizarian biomass distribution, we used the *'sample'* function (implemented in R version 3.2.0) to obtain a random subset of our dataset and test the latitudinal pattern of this dataset against the original dataset. We selected five latitude intervals of 30° (between 90°N and 60°S) into each of which we randomly extracted 20 sampling stations with bootstrap resampling. Difference between the resampled dataset (n = 100 sampling stations) and the original entire dataset (n = 694 sampling stations) was tested with a non-parametric Mann-Whitney U Test and no significant differences were observed ($p = 0.155$).

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Extended Data Table 1 | Sampling cruise information, number of stations sampled, and of UVP5 deployments (e.g. profiles) used to generate the dataset analyzed in this study.

Sampling Cruise Name	Year	Chief Scientists	Biomes	(0-100 m)		(100-500 m)	
				Sampling Stations	Profiles	Sampling Stations	Profiles
BOUM	2008	T. Moutin	Mediterranean Sea* (MEDI)	183	183	151	151
KEOPS II	2011	S. Blain	Antarctic Biome (ANTA)	7	7	7	7
LOHAFEX	2009	V. Smetacek	Antarctic Biome (ANTA)	27	27	21	21
CCE-LTER	2008	M. Landry M. Ohman	California Upwelling Coastal* (CCAL)	75	75	58	58
M96	2013	J. Karstensen	Atlantic Trade Wind Biome (SATL)	60	77	58	59
MALINA	2009	M. Babin	Atlantic Polar Biome (ATPL)	119	119	54	54
MOOSE-GE	2012	L. Coppola	Mediterranean Sea* (MEDI)	87	87	79	79
MSM22	2012	P. Brandt	Atlantic Trade Wind Biome (SATL)	108	108	80	80
MSM23	2012	M. Visbeck	Atlantic Trade Wind Biome (SATL)	45	45	45	45
Tara Oceans	2009	*Tara* Oceans Consortium	Mediterranean Sea* (MEDI)	27	46	24	29
	2010		Indian Ocean Trade Wind Biome (IND)	23	94	22	80
	2010		Atlantic Trade Wind Biome (SATL)	16	82	14	55
	2011		Antarctic Biome (ANTA)	3	6	3	5
	2011		Pacific Trade Wind Biome (PAC)	34	218	33	140
	2011		California Upwelling Coastal* (CCAL)	4	30	4	26
	2012		Atlantic Trade Wind Biome (SATL)	2	17	2	13
	2012		Atlantic Westerly Winds Biome (NATL)	12	92	12	73
Tara Arctic	2013	*Tara* Oceans Consortium	Atlantic Westerly Winds Biome (NATL)	1	9	1	9
	2013		Atlantic Polar Biome (ATPL)	44	132	26	72
Total				877	1,454	694	1,056

Biogeochemical Biomes are defined according to ref. 29. *This province was treated separately from its biome since it showed a strong pattern in rhizarian abundance compared to the other provinces in the same biome.

Extended Data Table 2 | Respective contributions of Rhizaria and other zooplankton abundances to the zooplankton community (>600 µm) integrated for the upper 0-500 m of the water column.

Longhurst's Biogeochemical Biomes	Number of sampling stations	Number of profiles	Zooplankton community / Sampling station (ind m^{-2})	Contribution to zooplankton community (%)	
				Rhizaria	Other zooplankton
Atlantic Polar Biome (ATLP)	80	126	206,905	3	97
Antarctic Biome (ANTA)	31	33	126,802	4	96
Atlantic Westerly Winds Biome (NATL)	13	82	434,501	15	85
Atlantic Trade Wind Biome (SATL)	199	252	273,721	15	85
Pacific Trade Wind Biome (PAC)	33	140	354,035	35	65
Indian Ocean Trade Wind Biome (IND)	22	80	68,911	37	63
Mediterranean Sea* (MEDI)	254	259	21,269	47	53
California Upwelling Costal* (CCAL)	62	84	556,582	81	19
Average proportion				33	67

*This province was treated separately from its biome since it showed a strong pattern in rhizarian abundance compared to the other provinces in the same biome.

Extended Data Table 3 | Carbon conversion factors used to assess biomass for the rhizarian categories discriminated.

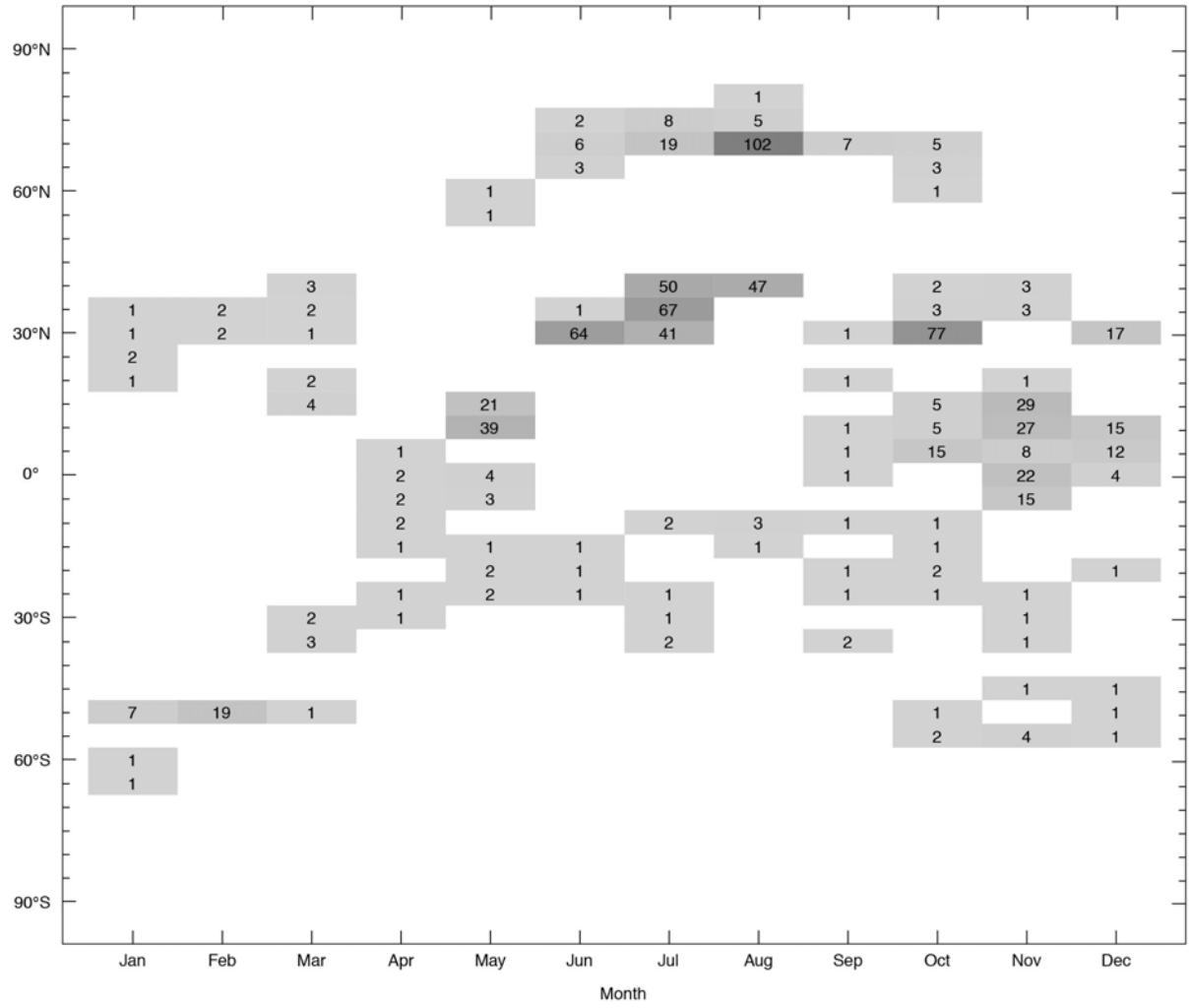
Category	Parameter estimated	Carbon conversion factors*	Conversion factors references
Acantharia (Acn)	Biovolume	0.0026 mgC mm^{-3}	(40)
Collodaria_colony (Col)	Surface Area	133 ngC cc^{-1}	(19,40)
Collodaria_solitary_black (SolB)	Biovolume	0.28 mgC mm^{-3}	(40)
Collodaria_solitary_fuzzy (SolF)	Biovolume	0.28 mgC mm^{-3}	(40)
Collodaria_solitary_globule (SolGlob)	Biovolume	0.009 mgC mm^{-3}	(40)
Collodaria_solitary_grey (SolG)	Biovolume	0.28 mgC mm^{-3}	(40)
Phaeodaria_leg (Phal)	Biovolume	0.08 mgC mm^{-3}	(41)
Phaeodaria_sphere_eye (PhaSe)	Biovolume	0.08 mgC mm^{-3}	(41)
Phaeodaria_sphere_thorn (PhaSt)	Biovolume	0.08 mgC mm^{-3}	(41)
Rhizaria_other (Rhiz)	Biovolume	0.08 mgC mm^{-3}	(41)

*Carbon contents are expressed as a function of the biovolume (mgC mm^{-3}) or as a function of the number of central capsules (cc) in colonial collodarian.

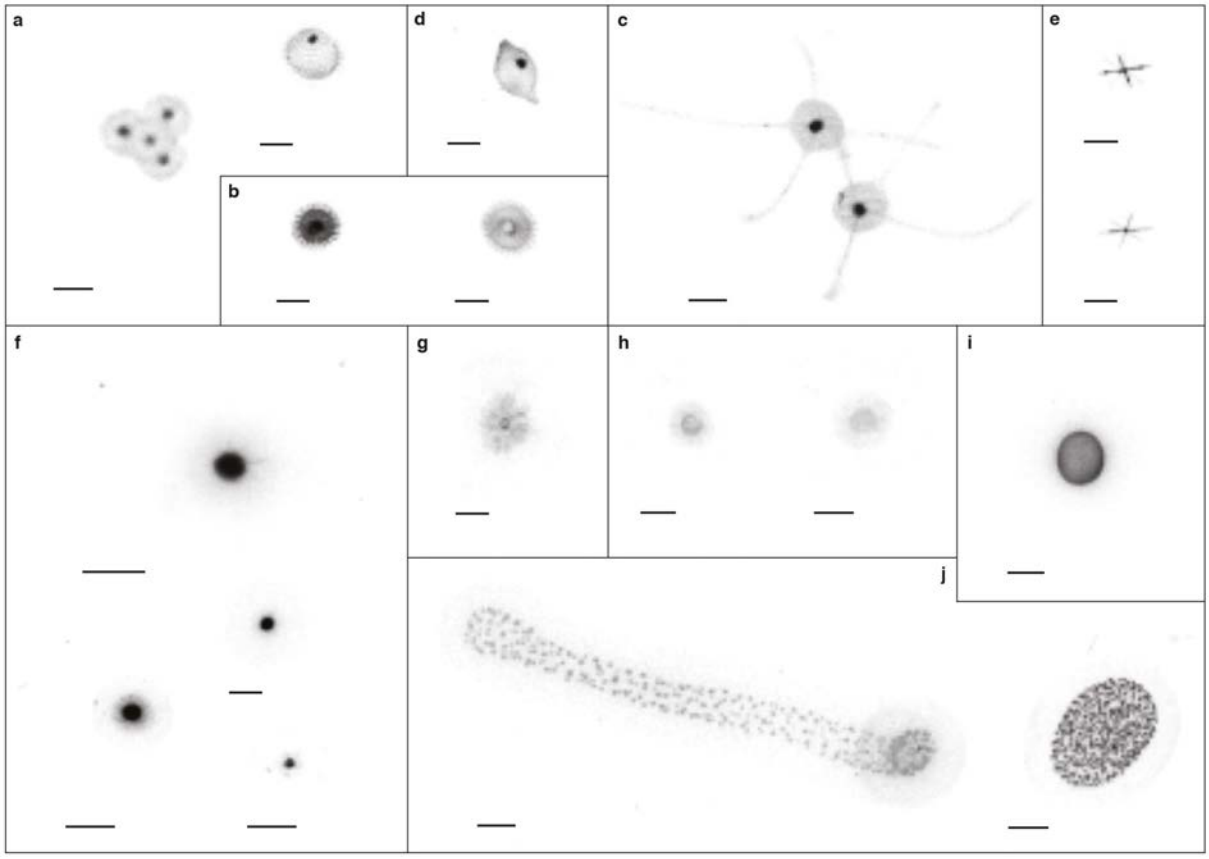
Extended Data Table 4 | Net primary production of photosymbiotic rhizarians (i.e. Collodaria and Acantharia) and their contribution to total and >2 µm net primary production in the global ocean and in the oligotrophic regions.

	NPP per surface (mgC d^{-1} m^{-2})			Contribution to global NPP (%)	Contribution in the oligotrophic regions (%)	
	Min	Max	Mean (± SEM)		To total NPP	To the >2 µm size fraction
Photosymbiotic Rhizaria (0-500 m)	0	5.64	0.26 (± 0.03)	0.071 (± 0.007)	0.18 (± 0.03)	0.59 (± 0.08)

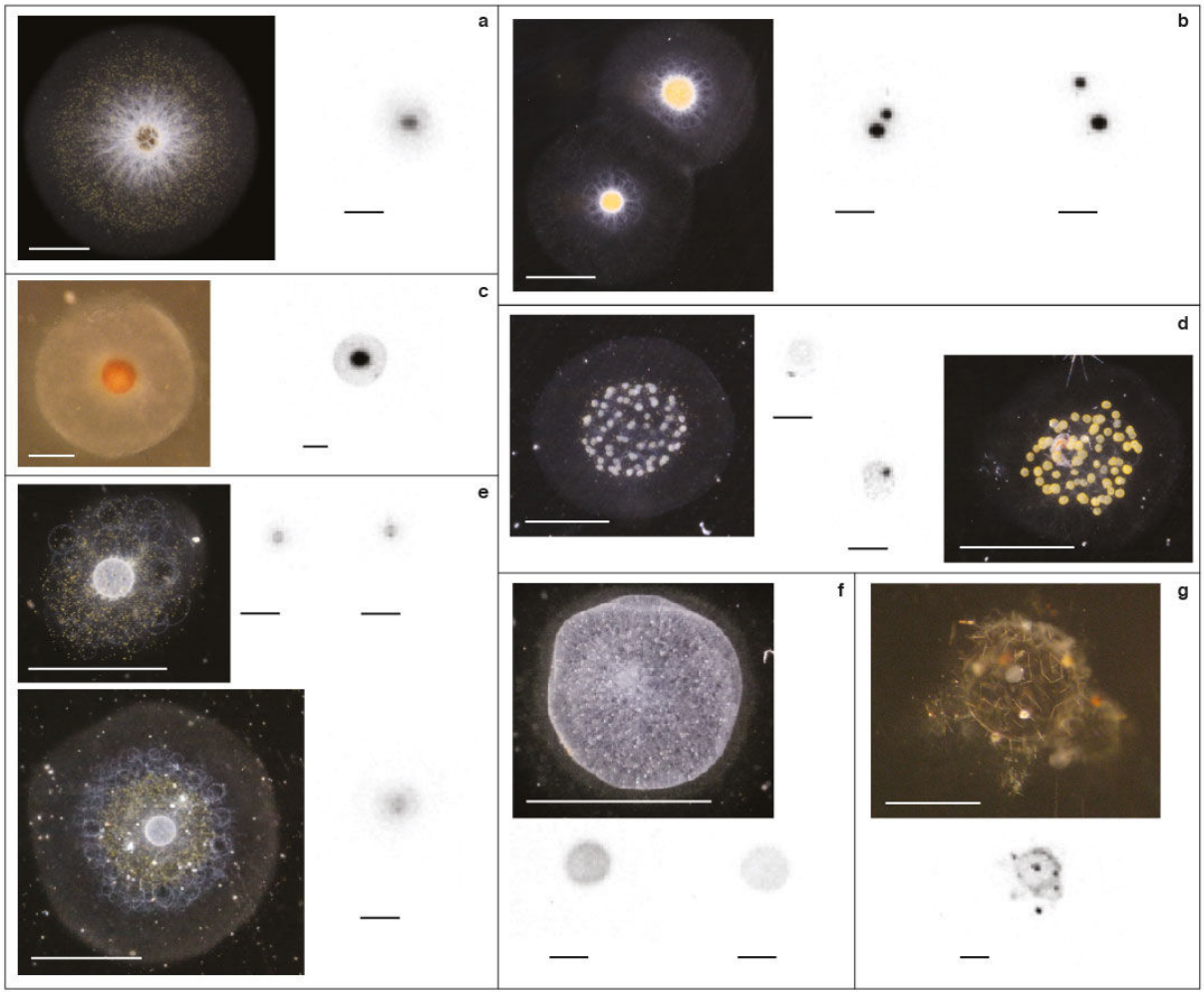
Global rhizarian NPP was derived from mean estimates (± standard error of the mean) assuming a total ocean surface of 3.61 x 10^{14} m^2 among which nearly 56% are considered oligotrophic (i.e. 2.04 x 10^{14} m^2; ref. 6). Rhizarian NPP contribution to global and oligotrophic regions was calculated based on total NPP estimates of 48.5 PgC and 11 PgC per year, respectively[38]. Contribution in the oligotrophic regions was estimated by extracting mean rhizarian NPP estimates in sampling stations where the Chla_{sat} was <0.1 mg m^{-3} (ref. 38). Contribution to the >2 µm size fraction assumed that pico-phytoplankton contributed up to 70% in oligotrophic regions[39].



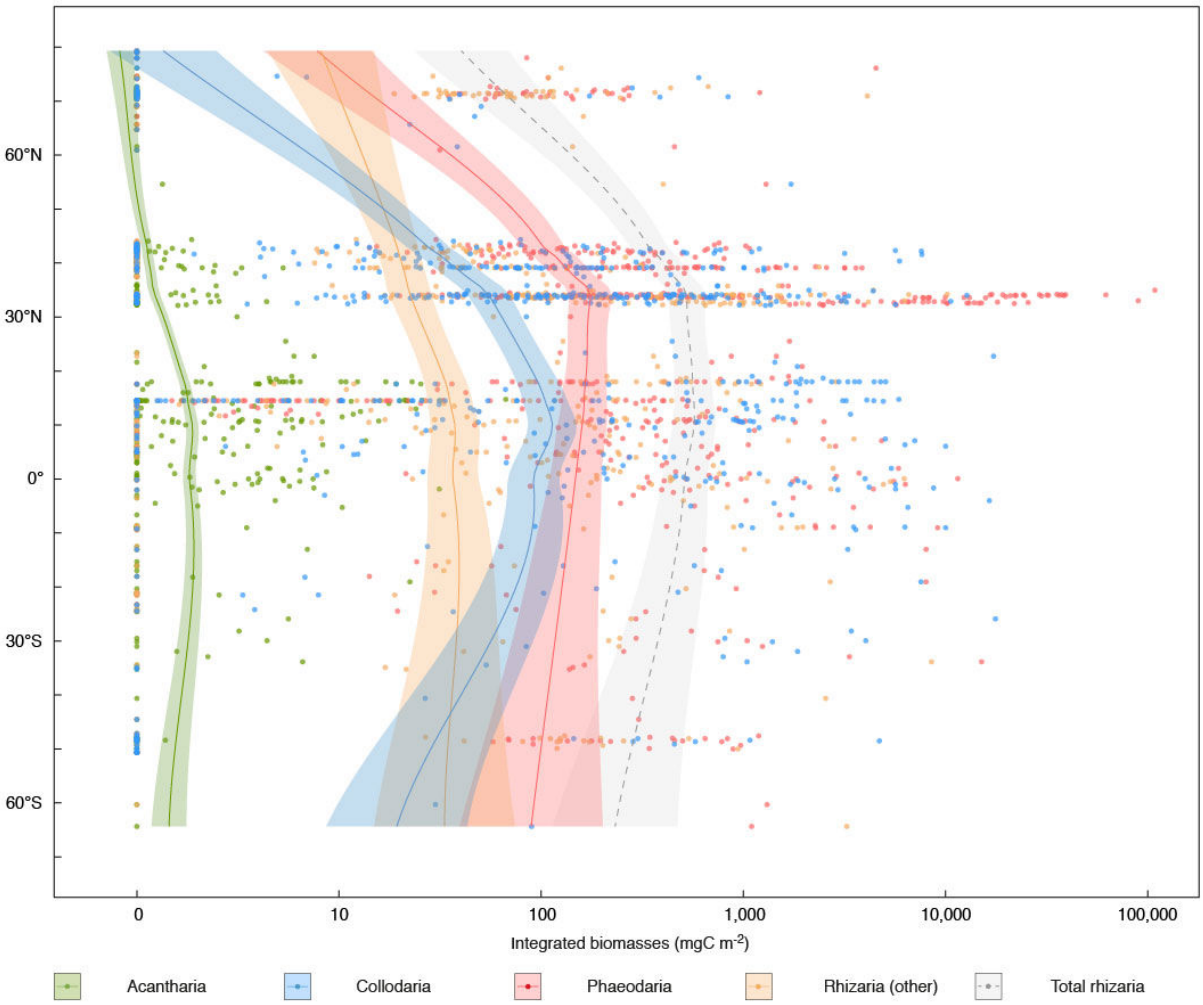
Extended Data Figure 1 | Sampling effort of the Underwater Vision Profiler surveys used in our study, represented across latitudes and months of the year. Rectangles identify latitude intervals of 5° affiliated at a given month. Numbers inside rectangles indicate the number of stations sampled (grey intensity is proportional to the number of stations).



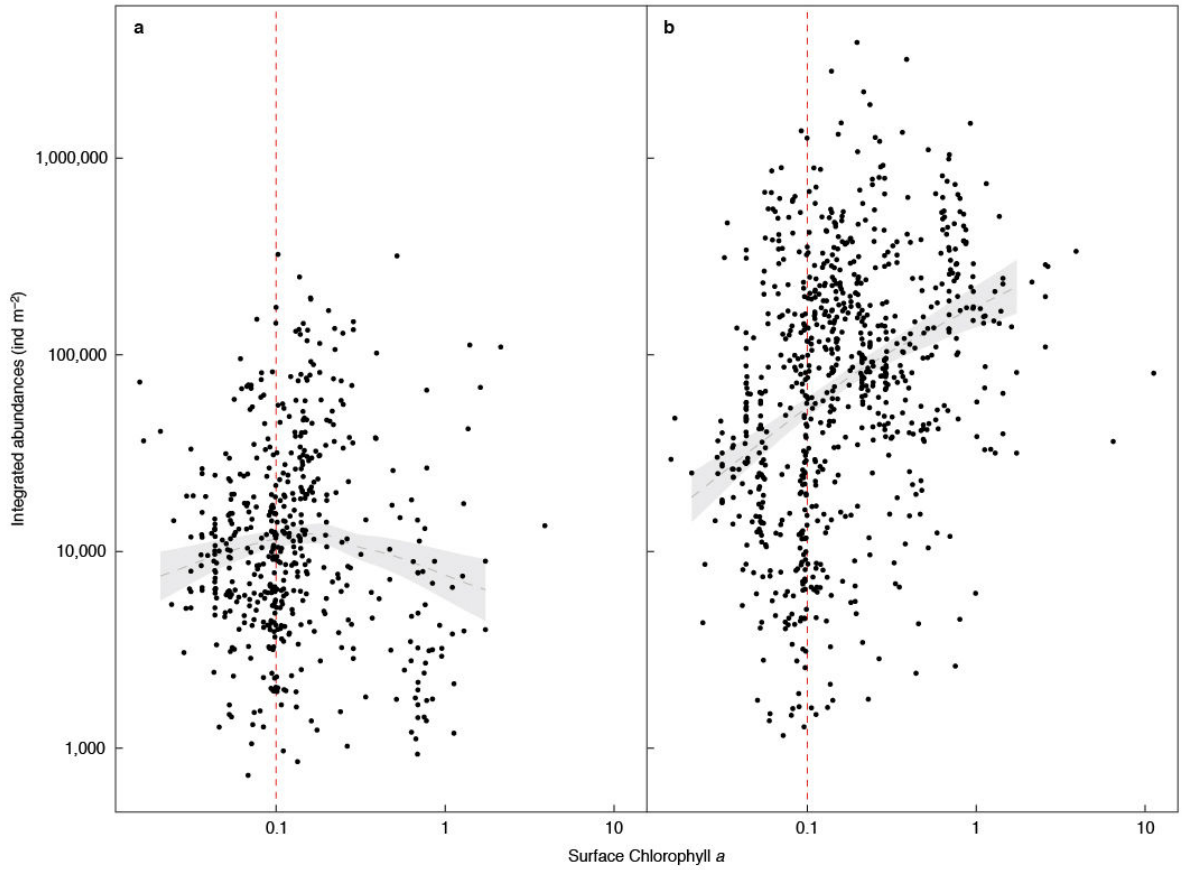
Extended Data Figure 2 | Images of the different rhizarian categories obtained with the UVP5. a-c, **Phaeodaria**: (**a**) phaeodarian spheres (PhaSe), (**b**) phaeodarian spheres with thorn edges (PhaSt) and (**c**) phaeodarians with long extensions (PhaL). **d**, **unidentified rhizarians** (Rhiz). **e**, **Acantharia** (Acn). **f-j**, **Collodaria**: (**f**) solitary collodarians with a dark central capsule (SolB), (**g**) solitary collodarians with a fuzzy central capsule (SolF), (**h**) solitary collodarians with a grey central capsule (SolG), (**i**) solitary collodarians with a globule-like appearance (SolGlob) and (**j**) colonial collodarians (Col). Detailed descriptions of the different categories are provided in the Methods section 'Refining the rhizarian images categories'. Scale bars = 2 mm.



Extended Data Figure 3 | Calibration of rhizarian categories through comparison of single specimen images acquired by UVP5 and optical microscopy. Optical microscopy images and UVP5 images were obtained from the exact same specimens. **a**, *Thalassicolla caerulea* (SolB). **b**, **c**, unidentified solitary collodarian species with dark central capsules (SolB). **d**, small collodarian colonies (Col). **e**, *Procyttarium primordialis* two solitary collodarians with a white central capsule (SolG). **f**, *Physematium muelleri* a solitary collodarian with a granular and opaque surface (similar to SolG). **g**, Phaeosphaeridae Phaeodaria (PhaSe). Scale bars = 2 mm.



Extended Data Figure 4 | Latitudinal biomass distribution (mgC m^{-2}) of the different rhizarian taxa identified (i.e. Acantharia, Collodaria, Phaeodaria and other Rhizaria) integrated over the upper 500 m of the oceans. Loess regressions with polynomial fitting were computed to illustrate the latitudinal trends. Pale areas display a 0.95 confidence interval around the trends.



Extended Data Figure 5 | Variation of UVP5 depth integrated abundances (0-100 m depth) as a function of the surface chlorophyll *a* extracted from satellite data. a. Integrated abundance of photosymbiotic Rhizaria (Collodaria and Acantharia). **b**. Integrated abundance of other zooplankton (including asymbiotic Rhizaria). The dotted red line indicates the threshold value used to characterize oligotrophic waters (Chla_{sat} = 0.1 mg m^{-3}; ref. 38). Loess regressions with polynomial fitting were computed to illustrate the pattern observed. Pale areas display a 0.95 confidence interval around the trends.

DISCUSSIONS ET PERSPECTIVES

« Instead of gazing down through water buckets and glass-bottomed boats, in addition to watching the fish milling about in aquariums, get a helmet and make all the shallows of the world your own. Start an exploration which has no superior in jungle or mountain; insure your present life and future memories from any possibility of ennui or boredom, and provide yourself with tales of sights and adventures which no listener will believe - until he too has gone and seen, and in turn has become an active member of the Society of Wonderers under-sea. »

William Beebe (1934)

1. Apports et limitations de la taxinomie intégrative

La classification des Collodaires a été entièrement réévaluée en choisissant une approche intégrant à la fois des critères morphologiques et des données moléculaires (Chapitre I). Cette nouvelle classification a permis de mettre en évidence de nombreux conflits entre les deux approches, suggérant que l'utilisation d'une seule méthode à la fois est parfois insuffisante pour correctement identifier les espèces, genres ou familles de Collodaires. Au-delà des questions purement taxinomiques, cette méthode nous a également permis de répondre à des questions d'ordre écologique.

1.1. Vers un nouveau schéma de classification

Lors des premières tentatives de classification des Collodaires, Karl Brandt, Ernst Haeckel, Roger Anderson ou Neil Swanberg soulignèrent tous leurs difficultés à trouver des critères taxinomiques fiables pour distinguer les Collodaires, en particulier ceux dépourvus de structure siliceuse. L'apport des outils moléculaires fut particulièrement bénéfique lors de notre réévaluation de classification des espèces de Collodaires « nus ». Pour ces espèces, l'identification taxinomique sur la base des observations faites en microscopie optique, n'a fourni que très peu de critères permettant de distinguer les différentes espèces. De plus, l'identification de spécimens préservés dans de l'éthanol, et non pas examinés vivants dès leur collecte, a eu tendance à altérer de façon non négligeable les quelques rares critères morphologiques (ex : forme des capsules centrales, positions des photosymbiontes). Néanmoins, grâce à l'analyse phylogénétique simultanée des gènes ribosomaux 18S et 28S, sept clades distincts ont pu être différenciés au sein de ces Collodaires « nus ». En particulier, cette approche a confirmé l'existence des Collophidiidae Biard et Suzuki (2015), une famille de Collodaires dont la création avait été suggérée à plusieurs occasions (Anderson et al., 1999 ; Zettler et al., 1999 ; Ishitani et al., 2012). Cependant, malgré la distinction génétique de ces différents clades, aucun caractère morphologique distinctif n'a pu être observé, notamment au sein des clades C7, C8, C10 et C11, où seules deux espèces du genre *Collozoum* ont pu être associées aux différentes entités génétiques (Chapitre I – Figure 1). Couplée aux analyses phylogénétiques, l'utilisation de méthodes alternatives de microscopie, notamment la microscopie électronique à transmission, devrait permettre de définir de nouveaux critères morphologiques, comme ce fut le cas lors de la reconnaissance du genre *Collophidium* (ex : organisations des parties intracapsulaires différentes entre *Collozoum* et *Collophidium* Anderson et al., 1999).

La classification morphologique des Collodaires possédant des structures cristallines, globalement plus aisée, doit néanmoins son succès à la mise au point d'un protocole simple visant à récupérer, au cours de l'extraction de l'ADN, les structures silicifiées présentes dans la matrice gélatineuse de ces Collodaires (Figure 19). Là où l'observation de la morphologie des squelettes et des spicules de Collodaires est souvent rendue délicate par la matrice gélatineuse, l'analyse en microscopie électronique à balayage offre un accès à des détails morphologiques invisibles en microscopie optique. Les résultats de ces analyses fines, une fois comparés à ceux obtenus par la phylogénie, se sont révélés être beaucoup résolutifs que

ceux obtenus en microscopie optique, soulignant ainsi la robustesse et la résolution de cette méthodologie. Cette méthode de récupération des squelettes s'est également avérée efficace pour d'autres groupes de Radiolaires, notamment les Nassellaires et les Spumellaires. Elle devrait permettre de rapprocher considérablement deux communautés de scientifiques, les micropaléontologues et les biologistes marins, toutes deux travaillant pourtant sur les mêmes organismes mais partageant rarement leurs sujets d'étude. Grâce à ce nouveau protocole (Figure 19), une révision de la classification morpho-moléculaire des Nassellaires est en préparation, publication pour laquelle j'ai réalisé des clichés préliminaires en microscopie électronique à balayage des squelettes (Pillet et al., *in prep*).

En dépit des bénéfices évidents d'un recours à la classification moléculaire, ces méthodes peinent parfois à distinguer deux espèces (identifiées morphologiquement) sur la seule base de leurs différences génétiques. Le choix des deux gènes ribosomaux, 18S et 28S, utilisés dans cette phylogénie, a été motivé par la présence d'une base de référence préexistante, bien peu exhaustive, mais également en raison de la nature de ces deux gènes, couramment utilisés en phylogénie moléculaire (Pawlowski et al., 2012), et aussi par l'importance de créer une base de données de référence permettant l'interprétation des études environnementales de *metabarcoding* (ex : Expédition *Tara* Océans). Pourtant, même si le gène 28S s'est révélé le plus résolutif, ces deux gènes ribosomaux n'ont pas montré la même efficacité à séparer les différents taxons au sein des trois familles de Collodaires (Figure 20). Au sein des différentes familles, les Collosphaeridae sont aisément différenciés grâce à l'utilisation de ces deux gènes, mais ce n'est pas le cas pour les Sphaerozoidae ou les Collophidiidae. L'utilisation de ces gènes dans les phylogénies moléculaires limite ainsi grandement la résolution

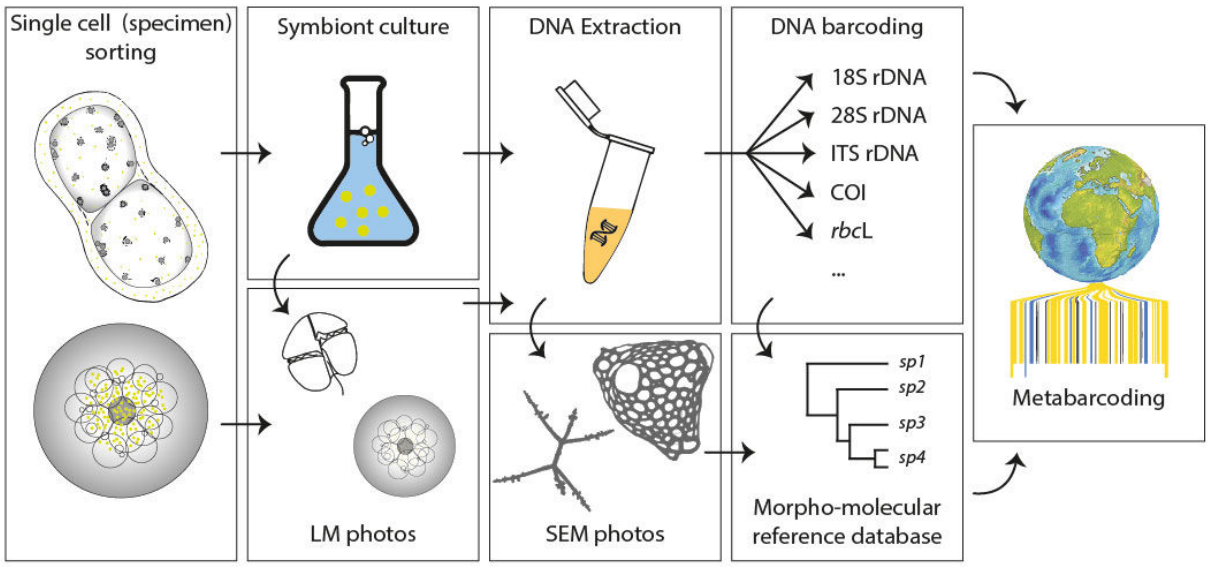


Figure 19 | Organigramme des différentes étapes conduisant de la cellule unique au *metabarcoding* *metabarcoding*. Chaque spécimen ou cellule unique est collecté et photographié. L'ADN de chaque spécimen est ensuite extrait et les structures siliceuses récupérées pour être imagées en microscopie électronique à balayage. L'amplification de gènes multiples permet la construction de phylogénies moléculaires agrémentées des analyses morphologiques. L'élaboration de bases de références permet par la suite d'explorer la diversité environnementale grâce au *metabarcoding*. Dans le cas des espèces photosymbiotiques, la mise en culture, la caractérisation morphologique et génétique, permettent de caractériser les partenaires symbiotiques. Modifié d'après Pawlowski et al. (2012).

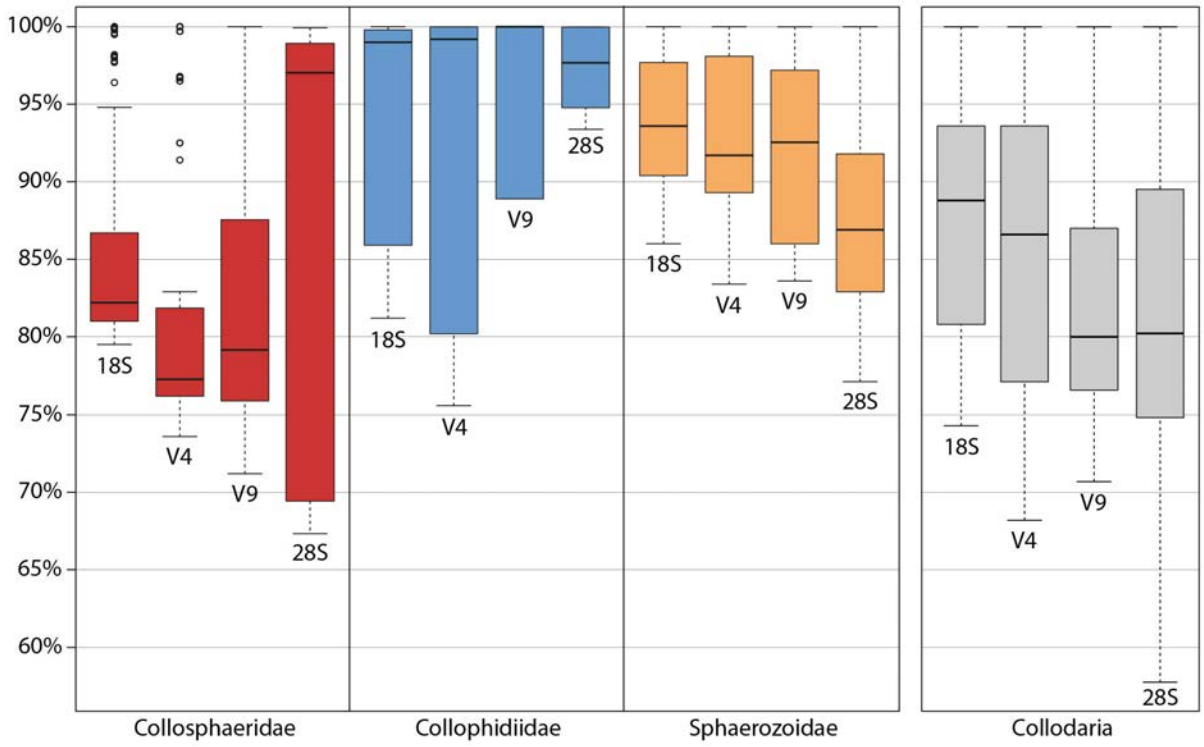


Figure 20 | Divergence interspécifique (pourcentage) pour le gène 18S RNA, ses régions hypervariables V4 et V9, ainsi que le gène 28S. La divergence est présentée au sein de chaque famille (gauche) et pour les Collodaires dans leur ensemble (droite).

taxinomique et malgré leur capacité à différencier les différentes familles de Collodaires, ces deux gènes ont des résolutions intergenres et interspécifiques limitées. L'utilisation de gènes tels que l'ADNr 5.8S, les espaces intergéniques (ITS1 et ITS2) ou l'ADNm Cox1, généralement considérés comme plus résolutifs que les marqueurs traditionnels ADNr 18-28S (Hebert et al., 2003; Pawlowski et al., 2012; Stern et al., 2012; Wang et al., 2015), devrait permettre d'affiner la résolution spécifique et d'améliorer la classification moléculaire des Collodaires, tout en fournissant un outil de travail plus exhaustif pour les futures études visant à explorer la biodiversité des Collodaires. Etant donné l'existence de quelques séquences de référence pour les espaces intergéniques, celles-ci pourraient fournir une base de travail pour intégrer de nouvelles séquences des espaces intergéniques de Collodaires.

1.2. Quand la taxinomie intégrative offre un aperçu du cycle de vie

Les méthodes de *barcodes* moléculaires peuvent également parfois se révéler être des approches utiles pour mieux comprendre le cycle de vie de certains groupes planctoniques (Lindeque et al., 2012; Decelle et al., 2013). Ceci s'est illustré chez les Collodaires avec la similarité génétique observée entre spécimens coloniaux et solitaires, ces derniers étant jusqu'à présent considérés comme formant une famille à part entière (Thalassicollidae ; Chapitre I). Même si leurs morphologies sont diamétralement opposées, les individus solitaires séquencés ont montré une identité génétique quasi parfaite avec des spécimens coloniaux. Si certains spécimens solitaires ont pu être reliés à une forme coloniale (ex :

Thalassophysa pelagica et *Collozoum pelagicum*), de nouvelles séquences de Collodaires solitaires devront être acquises pour établir des liens formels entre ces différentes formes.

Si les analyses phylogénétiques ont enfin pu relier les deux morphologies principales de Collodaires, cette identité commune avait déjà été suggérée auparavant grâce au suivi *in vitro* de plusieurs spécimens (Brandt, 1885 ; Hollande et Enjumet, 1953). Cette même identité génétique apporte donc de nouveaux éléments dans la compréhension du cycle de vie des Collodaires, qui n'a pourtant jamais été élucidé en plus d'un siècle et demi de recherches. Bien que cette question n'ait pas été spécifiquement abordée au cours de cette thèse, les nombreuses heures d'observations qui ont pu être réalisées *in vivo* sur des Collodaires ont contribué à documenter certains aspects du cycle de vie des Collodaires (Figure 21). Ainsi, le cycle de vie hypothétique des Collodaires se rapproche d'un cycle de vie haplo-diplophasique (alternance des phases haploïdes et diploïdes) hétéromorphe (présence de deux morphotypes distincts) où la reproduction sexuée et asexuée peuvent alterner au sein de chaque phase (Figure 22). Même si la ploïdie des différentes phases observées n'est pas formellement démontrée à ce jour, l'existence de l'alternance haploïde/diploïde est motivée par la nature des mécanismes cellulaires précédant la formation des *swarmers* où la multiplication massive des noyaux (mitose) dans la(les) capsule(s) centrale(s) suggère un passage de la cellule mère (diploïde) vers le(s) *swarmer*(s) haploïde(s). Néanmoins, il conviendrait d'étudier spécifiquement la ploïdie des formes solitaires et coloniales ainsi que des swarmers afin de confirmer cette hypothèse. Par exemple, l'utilisation de la PCR quantitative (qPCR) pour estimer le nombre de copies de gènes spécifiques, exprimés une seule fois, permettrait de comparer la ploïdie des *swarmers* et des formes solitaires ou coloniales. Pourtant, quand bien même leur cycle de vie semble se préciser, il reste encore une part d'ombre importante dans le cycle de vie des Collodaires, où les swarmers semblent notamment être le lien manquant entre les deux formes solitaires et coloniales lors de la phase sexuée. Elucider des questions telles que les mécanismes écologiques, qui induisent un changement de phase dans le cycle de vie ou le devenir des swarmers, permettrait de mieux appréhender l'écologie de ces organismes. Au-delà d'un aspect purement écologique, comprendre le cycle de vie des Collodaires apparaît comme un prérequis pour de futures tentatives de mise en culture, étape fondamentalement cruciale pour pouvoir étudier les Collodaires sur le long terme.

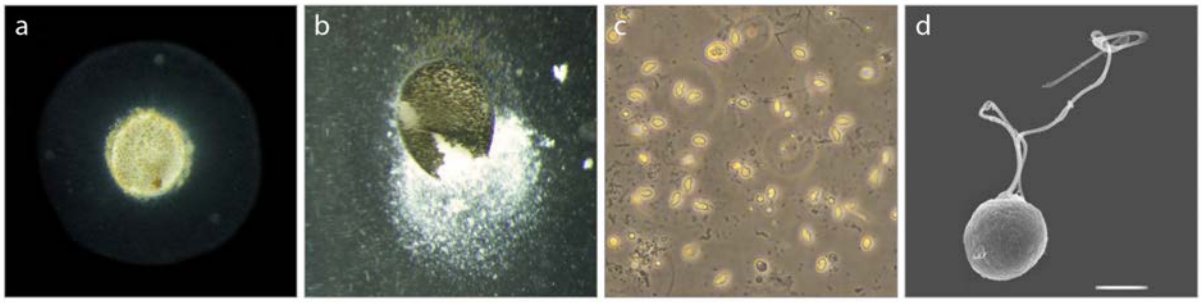


Figure 21 | Production des *swarmers* chez un Collodaire solitaire. (a) Un spécimen solitaire précédent la production de swarmer. La capsule centrale s'agrandie et devient de couleur pâle. (b) La capsule centrale sédimente, s'ouvre et (c) libère une multitude de *swarmers*. (d) Détail en microscopie électronique d'un *swarmer* de Collodaire (Yuasa et Takahashi, 2014).

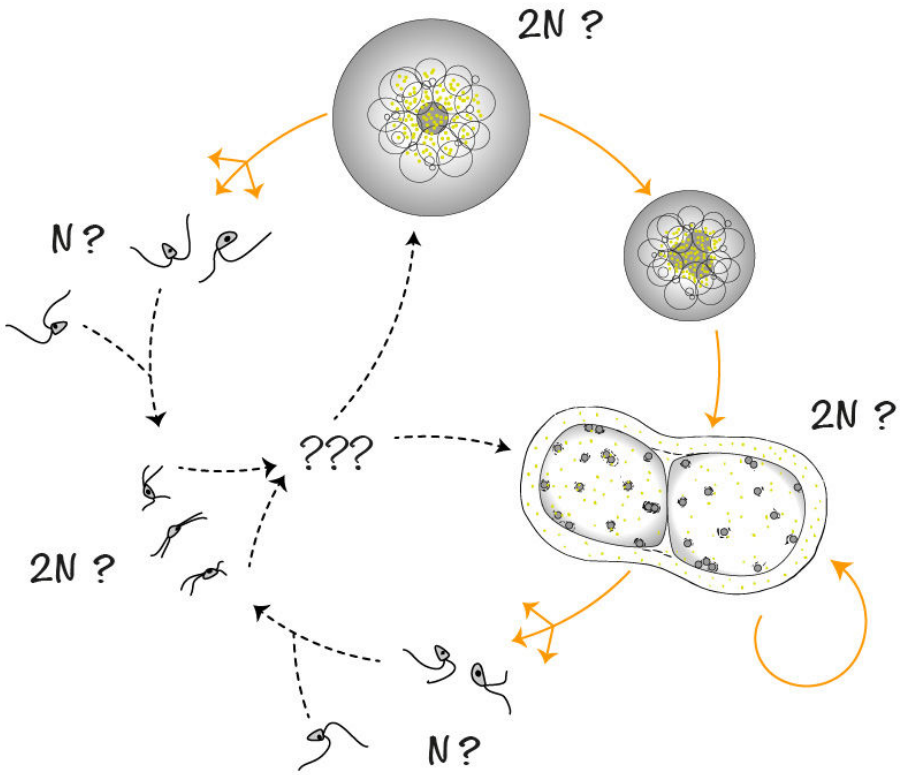


Figure 22 | État des connaissances sur le cycle de vie des Collodaires. Les flèches orange illustrent des processus observés et documentés pour certains au cours de cette thèse. Les flèches en pointillés soulignent les liens manquants et hypothétiques. Une ploïdie est proposée pour chaque forme.

2. Vers une nouvelle histoire évolutive des Collodaires ?

Les analyses phylogénétiques présentées dans le Chapitre I ont permis de confirmer la place des trois familles de Collodaires et d'étudier les relations évolutives qu'elles partagent. En raison de leur nature particulière (les seuls Radiolaires avec des espèces sans squelette et formant des colonies) et les difficultés à les collecter, les Collodaires ont rarement fait l'objet d'études quant à leur histoire évolutive.

2.1. Les spicules au cours de l'évolution

Les Collodaires comptent parmi l'un des derniers ordres de Radiolaires apparus au cours des temps géologiques (Suzuki et Oba, 2015). L'origine des Radiolaires polycystines remonte quant à elle à l'orée de l'ère primaire, soit il y a plus de 515 millions d'années, au Cambrien inférieur (Obut et Iwata, 2002). Les formes ancestrales des Radiolaires, certaines connues sous le nom d'Archaeospicularia ne présentaient pas la morphologie typique que l'on associe de nos jours à ces organismes, à savoir un squelette complexe, mais possédaient en revanche une multitude de spicules simples, très similaires à ceux des éponges (Dumitrica et al., 2000 ; Maletz, 2011). Ces spicules évoluèrent au cours du temps pour former peu à peu des structures cristallines plus complexes, dont le squelette sera l'aboutissement le plus complexe

(Campbell, 1954 ; Maletz, 2011). La présence de spicules au sein des Collodaires soulève donc de nombreuses questions vis-à-vis des processus évolutifs qui ont eu lieu au sein des Radiolaires pour mener jusqu'aux Collodaires.

À cause du manque de structures silicifiées chez certaines familles de Collodaires, les données fossiles de Collodaires sont assez rares. On daterait l'apparition des Collodaires au milieu du Paléogène, soit il y a environ 43 millions d'années (Haslett, 2004). Cette datation, effectuée à partir de microfossiles de Collosphaeridae, ne permet en aucun cas d'avoir une datation pour l'apparition des Sphaerozoidae et de leurs spicules. Cependant, grâce à la phylogénie établie dans le Chapitre I, il semblerait que Collosphaeridae et Sphaerozoidae aient co-évolué au cours du temps. Deux lignées s'opposent donc, d'un côté des Collodaires possédant un squelette, de l'autre des formes possédant des spicules ou même dénuées de toute structure minérale (Chapitre I – Figure 1). À cause de leur grande fragilité et de leur ressemblance avec les spicules d'éponges, il est peu probable que de futures données fossiles soient disponibles pour les Collodaires à spicules. L'interprétation de l'histoire évolutive des Collodaires repose donc uniquement sur les données fossiles de Collosphaeridae. Grâce aux calibrations chronostratigraphiques et à l'utilisation de la méthode des horloges moléculaires, il est possible d'estimer les temps de divergence à partir d'une phylogénie moléculaire (Ho, 2008). Une telle approche a déjà été tentée pour comprendre l'évolution des Collodaires (Ishitani et al., 2012). Cependant, cette tentative fut contrainte par une phylogénie trop peu résolutive et l'utilisation de données fossiles non validées. Dans le cadre d'une future collaboration avec le Dr. Noritoshi Suzuki, l'utilisation d'horloges moléculaires, grâce à la nouvelle phylogénie des Collodaires et le choix de données fossiles validées, permettra de fournir de nouveaux arguments pour étayer notre hypothèse (développée ci-dessous) concernant l'histoire évolutive des Collodaires.

Le schéma d'évolution proposé ici n'a pas vocation à être utilisé comme tel, mais doit plutôt former une base de réflexion sur l'évolution des Collodaires. Après avoir constitué un caractère ancestral, puis évolué au cours du temps, des spicules, très similaires à ceux des Archaeospicularia, réapparaissent au sein des Collodaires plusieurs centaines de millions d'années après avoir disparu de toute famille de Radiolaires. Il est peu probable que les spicules aient été conservés au cours de l'évolution tant il est impossible de trouver une telle structure dans les autres familles de Radiolaires. Si l'on considère le taux d'évolution des gènes 18S et 28S, la phylogénie (Chapitre I - Figure 1) semble indiquer que les spicules seraient apparus en même temps que les squelettes de Collodaires, soit au milieu de l'Eocène (~43 m.a.). Or, une étude récente a démontré que la taille et la silicification des squelettes de Radiolaires avaient considérablement diminué au cours du Cénozoïque (66 m.a. à maintenant) notamment aux basses latitudes où la concentration en silice chuta brutalement (Lazarus et al., 2009). Cette étude a suggéré que l'augmentation de la stratification des océans à cette époque pouvait avoir entraîné une diminution globale des concentrations de silice. De plus, l'essor des diatomées, plus compétitives que les Radiolaires dans l'assimilation de la silice, pourrait avoir participé à la baisse de silicification des squelettes de Radiolaires. C'est également à cette période que les données fossiles semblent indiquer l'apparition des Collodaires, parmi les seuls Radiolaires à ne plus posséder un squelette de silice. Ainsi, on peut émettre l'hypothèse que face à une diminution de la disponibilité en silice, les Collodaires constituent

une forme évoluée de Radiolaires, particulièrement adaptée aux basses latitudes, ayant réduit leur capacité d'assimilation de la silice au point de voir leur squelette régresser vers une forme primitive que constituent les spicules. Cette régression aurait également pu se poursuivre par la perte totale de structure silicifiée et contribuer à l'apparition des formes « nues » de Collodaires (ex : le genre *Collozoum*). Même si cette hypothèse semble pouvoir expliquer le changement de structure silicifiée chez les Collodaires, elle repose uniquement sur la comparaison entre deux des trois familles de Collodaires et ne prend pas en compte l'existence des Collophidiidae, exclusivement composés d'espèces « nues » et formant le clade sœur des Collosphaeridae.

2.2. Les Collophidiidae : vers une adaptation des Collodaires aux zones bathypélagiques ?

Longtemps passés inaperçus, voire ignorés des schémas de classification taxinomique, les Collophidiidae, décrits formellement au cours de cette thèse (Chapitre I), forment une famille de Collodaires pour le moins énigmatique. Pendant près d'un siècle, ces Collodaires ont été considérés comme faisant partie du genre *Collozoum*, certes à juste titre puisque leur morphologie « nue » est très similaire à celle des espèces du genre *Collozoum*. Rarement collectés, leur étude n'a permis de décrire que trois espèces coloniales à ce jour, *Collophidium serpentinum*, *Collophidium ellipsoides* et *Collophidium ovatum* (Haeckel, 1887), toutes trois abritant quelques photosymbiontes, identiques à ceux trouvés dans les autres familles de Collodaires (Probert et al., 2014). Bien que des Collophidiidae aient été observés à quelques rares occasions en surface (Chapitre II-1), une série d'études récentes (Edgcomb et al., 2011 ; Pernice et al., 2015) a permis de collecter des données dans les zones bathypélagiques des océans, où les Collophidiidae se sont révélés être particulièrement représentés (Chapitres I et II-1).

La surface des océans, ou zone épipélagique, ne représente certes qu'une part infime de l'océan mondial (moins de 2% de son volume) mais la diversité et l'abondance d'organismes qu'elle abrite ont fait l'objet de près de plus de la moitié des études recensées sur la base de données OBIS (Webb et al., 2010). L'océan profond, quant à lui, constitue le plus grand écosystème sur Terre et abrite une vaste biodiversité restant néanmoins trop peu étudiée, à cause notamment des difficultés à échantillonner cette zone (Robison, 2004 ; Robison, 2009 ; Webb et al., 2010). Dans ses surprenants comptes rendus de plongées en bathyscaphe, Grégoire Trégouboff apportera de précieuses observations des eaux profondes de la Baie de Villefranche-sur-Mer, parmi lesquelles il mentionnera la première observation de Collodaires coloniaux à de telles profondeurs (Trégouboff, 1956, 1958, 1959). Plusieurs décennies plus tard, des Collodaires vont de nouveau réapparaître dans les profondeurs des océans, mais cette fois-ci sous la forme de séquences d'ADN (López-García et al., 2001 ; Countway et al., 2007 ; Not et al., 2007 ; Edgcomb et al., 2011 ; Pernice et al., 2015). Un tel constat nous oblige à revoir complément notre compréhension de l'écologie des Collodaires, jusqu'alors uniquement observés dans les zones photiques et en association obligatoire avec des photosymbiontes pour l'ensemble des espèces décrites à ce jour. Ces séquences issues du milieu profond ne nous renseignent nullement sur la morphologie des organismes et on ne

peut que spéculer sur la forme des Collodaires présents à de telles profondeurs. Au-delà de la forme typiquement coloniale qu'a pu observer Grégoire Trégouboff, l'existence des swarmers portant la signature moléculaire des Collodaires dans les zones bathypélagiques pourrait biaiser notre interprétation. La quasi-totalité des séquences extraites de ces données profondes suggère que seuls les Collophidiidae sont extraits à de telles profondeur (Chapitre I - Figure 4). Or, si la signature moléculaire des Collodaires profonds doit son origine à de tels swarmers, l'ensemble des trois familles devrait être représenté puisque, en effet, la production de swarmers est un phénomène observé dans toutes les familles.

Afin de compléter nos connaissances sur ce qui pourrait être désormais considéré comme une communauté profonde de Collodaires, il convient de mettre en place une stratégie d'échantillonnage visant à collecter spécifiquement ces organismes. Un tel prélèvement devra se faire de façon très délicate afin de collecter des échantillons de plancton intacts. L'utilisation de filets fermants (ex : MOCNESS) devrait permettre la collecte de ces Collodaires profonds. Néanmoins, la collecte d'échantillons d'eau de mer prélevés à la bouteille Niskin, semble être nécessaire dans l'éventualité où la signature moléculaire des Collodaire doive son origine aux swarmers. Au-delà d'apporter une contribution significative aux connaissances sur les Collodaires, la présence de Collodaires à de telles profondeurs pourrait également soulever l'existence de Collodaires asymbiotiques. En outre, si seuls les Collophidiidae constituent une communauté profonde, n'auraient-ils pas pu évoluer spécifiquement pour s'adapter à une niche écologique radicalement différente de celle des Collodaires de surface ?

2.3. Collodaires et colonialité

Dans son article au titre évoquant une *« course à l'armement »*, Victor Smetacek (2001) évoque l'évolution des différentes mesures défensives au sein du phytoplancton lui permettant de s'adapter à la pression trophique exercée par ses prédateurs. Que ce soit les diatomées et leurs frustules, les tintinides et leurs lorica (terme emprunté aux armures portées par les soldats romains), ou même les Radiolaires et leur squelette, toutes ces morphologies seraient autant de formes permettant une meilleure protection contre les prédateurs (Hamm et Smetacek, 2007 ; Porter, 2011). Dans ce contexte, il est donc particulièrement intéressant de voir que l'évolution (ou régression) du squelette chez les Collodaires vers des formes moins complexes, en comparaison des autres ordres de Radiolaires, correspond également à l'apparition de la colonialité. Bien que l'apparition des colonies chez les Collodaires ait également concerné les espèces possédant un squelette (Collosphaeridae), cette organisation des cellules sous forme coloniale est propre aux Collodaires (Suzuki et Not, 2015). Même si l'ensemble des Radiolaires est constitué d'organismes solitaires, le stade solitaire observé chez les Collodaires est particulier du fait de sa taille démesurée et de sa morphologie où la cellule est entourée d'une matrice gélatineuse faisant près de 2 à 4 fois sa taille (Anderson, 1983). Il se pourrait alors que les Collodaires aient compensé la perte de structure siliceuse au profit d'une adaptation vers la colonialité, un gigantisme leur conférant la protection nécessaire contre leurs prédateurs. Enfin, en plus d'offrir une protection certaine face à des prédateurs, le colonialisme permet à une multitude de cellules de partager un seul et même

microenvironnement, où les échanges peuvent être favorisés, et où la colonie elle-même peut créer des conditions optimales propices au maintien et au développement de ses partenaires photosymbiontes.

2.4. La photosymbiose, facteur de distribution des Collodaires ?

L'ensemble des Collodaires décrits à ce jour, y compris les Collophidiidae, sont caractérisés par l'existence d'une photosymbiose obligatoire (Hollande et Enjumet, 1953). Si la présence de micro-algues a été révélée dès les premières observations de Collodaires (Brandt, 1882a, 1882b ; Haeckel, 1862, 1887), l'identité de ces photosymbiontes n'a jamais pourtant été démontrée. Grâce à la collecte de nombreux spécimens de Collodaires au cours de cette thèse, des analyses détaillées ont permis de caractériser l'identité génétique et morphologique des photosymbiontes de Collodaires, identifiés comme l'unique espèce *Brandtodinium nutricula* (Probert et al., 2014 ; Annexe 3). La présence systématique d'une seule et unique espèce de photosymbiontes au sein de l'ensemble des espèces étudiées renforce donc l'aspect spécifique de la relation entre les Collodaires et leurs partenaires symbiotiques. La nature exclusive de cette photosymbiose contraste avec la plus grande diversité taxonomique des partenaires symbiotiques chez les autres Radiolaires (ex : Acanthaires ; Decelle et al., 2012a, 2012b) ou chez les Foraminifères (Decelle et al., 2015). Néanmoins, il y a encore un manque flagrant de données permettant de comprendre la nature de ces relations. S'agit-il d'un pur mutualisme, où l'hôte Collodaire fournit un microenvironnement favorable à ses photosymbiontes qui lui offrent un apport nutritif en retour (Anderson, 1976, 1983), ou bien s'agit-il d'un parasitisme « inversé » où seul l'hôte bénéficie de cette association (Decelle, 2013) ?

De plus, il a été démontré qu'au cours du cycle de vie des Collodaires, les juvéniles (ex : les proto-colonies) n'héritent pas des symbiontes de la cellule-mère (transmission verticale) mais doivent acquérir leurs photosymbiontes directement dans l'environnement (transmission horizontale ; Anderson, 2012). Comprendre la nature de ces interactions permettrait de mieux appréhender la manière dont les photosymbiontes sont recrutés dans l'environnement. La persistance de cette transmission horizontale des symbiontes suggère que les populations de Collodaires sont d'une certaine façon contraintes par les populations des photosymbiontes vivant en phase libre. Grâce aux données de *metabarcoding* et d'hybridation *in situ* (FISH) acquises lors de la série saisonnière dans la Baie de Villefranche-sur-Mer (Chapitre II-2), il serait possible d'examiner la cooccurrence entre les symbiontes en phase libre et leurs hôtes. En outre, grâce à l'aspect saisonnier de ces prélèvements, il serait possible de voir en détail comment évoluent les deux populations au cours du temps. La communauté de Collodaires est-elle plus influencée par une série de variables environnementales ou est-elle sous l'influence de la population de photosymbiontes en phase libre et de leurs disponibilités pour les populations de juvéniles ?

Le succès écologique des Collodaires semble désormais être étroitement lié à une série d'évolutions parmi lesquelles la photosymbiose pourrait contraindre significativement la persistance d'une population donnée. L'histoire évolutive des Collodaires suggère qu'ils ont évolué au cours des derniers millénaires dans des zones de plus en plus oligotrophiques, et ceci grâce à l'apport nutritif de leurs symbiontes, qui, à l'échelle d'une colonie, pourrait

ressembler à de véritables « microfermes marines ». Les Collodaires auraient donc pu s'adapter à ces zones où la nourriture est rare et particulièrement disputée. De manière générale, il a été suggéré que la photosymbiose permettrait aux grands organismes, tels que les Rhizaria, de survivre dans ces milieux hostiles (Norris, 1996 ; Stoecker et al., 2009). Il apparaît donc que la photosymbiose pourrait être un moteur expliquant les patrons de distribution de biomasse et de diversité observés au cours de cette thèse.

3. Nouvelles approches pour l'étude de la biodiversité et l'écologie des Collodaires

Pendant près d'un siècle de recherches océanographiques, les Collodaires ne sont apparus qu'à de très rares occasions dans les études portant sur la diversité et l'abondance du plancton dans l'océan mondial. Le choix des outils utilisés tout au long de cette thèse est le fruit d'une réflexion sur la meilleure façon d'échantillonner ces organismes fragiles que sont les Collodaires. Après des décennies passées à utiliser des techniques d'échantillonnage traditionnelles (ex : filets à plancton, bouteilles Niskin), le développement récent de nouvelles technologies commence à changer peu à peu notre vision des océans en démontrant l'importance de compartiments planctoniques longtemps ignorés. Ainsi, au cours de cette thèse, l'utilisation de méthodes d'étude alternatives, telles que le *metabarcoding* (Chapitre II) ou l'imagerie *in situ* (Chapitre III), a permis de s'affranchir de ces techniques, souvent proposées pour expliquer la sous-représentativité des Collodaires du fait des difficultés à les identifier ou de leur fragilité limitant nos capacités à les collecter.

3.1. L'apport de l'imagerie *in situ*

Une partie des études réalisées au cours de cette thèse se sont appuyées sur la base de données immense que constitue la collection d'images *in situ* acquises par l'Underwater Vision Profiler (UVP), un système d'imagerie *in situ* développé au Laboratoire d'Océanographie de Villefranche-sur-Mer depuis 1992. Grâce à l'utilisation d'une partie des données (1 450 profils verticaux sur les 5 000 disponibles à l'heure actuelle), ces travaux ont mis en évidence que les Collodaires étaient des acteurs importants des communautés zooplanctoniques, en particulier dans les zones oligotrophiques (Chapitre III). Même si l'utilisation de l'UVP a fourni quantité d'informations déterminantes sur l'abondance et la biomasse des Collodaires dans de nombreuses régions des océans, ces observations sont néanmoins restreintes à la gamme de taille de l'instrument (600 µm ~ 10 mm). De plus, il semblerait que l'UVP, dans sa configuration actuelle, ne puisse pas correctement échantillonner les premiers mètres de la colonne d'eau, à la surface des océans. Or, c'est dans cette couche de surface que plusieurs études ont décrit de très fortes densités de Collodaires (Swanberg, 1979 ; Caron et Swanberg, 1990 ; Caron et al., 1995 ; Michaels et al., 1995). Ces agglomérations de sub-surface semblent certes être purement liées à des mécanismes physiques (ex : stabilité de la colonne d'eau, météo clémente, cellules de Langmuir, etc.) et ne concernent que certaines zones océaniques (typiquement les grands gyres), mais le sous-échantillonnage d'une telle masse de Collodaires

pourrait biaiser toute estimation globale d'abondances ou de biomasses. Avec la gamme grandissante de systèmes d'imagerie *in situ* développés récemment (Wiebe et Benfield, 2003 ; Benfield et al., 2007), plusieurs alternatives s'offrent à l'exploration *in situ* des Collodaires. Parmi celles-ci, l'*IS*IIS (*In Situ* Ichtyoplankton Imaging System ; Cowen et Guigand, 2008) est un instrument avec un très fort potentiel pour prolonger l'exploration *in situ* des Collodaires. Avec sa résolution et son volume échantillonné, tous deux supérieurs à l'UVP, l'*IS*IIS a permis d'imager quantité de Collodaires, dont de très grandes colonies que l'UVP n'a souvent pas pu capturer (Figure 23). De plus, grâce à son design qui permet d'enregistrer des images en continu le long de transects, l'*IS*IIS permet de mener des analyses avec une résolution spatio-temporelle bien supérieure à celle de l'UVP (Luo et al., 2014 ; Greer et al., 2015). Malgré cet avantage conséquent, l'*IS*IIS est un instrument qui suppose une logistique importante en raison de sa taille imposante, et son utilisation ne permet pas de répondre aux mêmes questions scientifiques que l'UVP, qui lui est plus simple à mettre en œuvre lors d'une expédition océanographique. Enfin, l'UVP comme, a fortiori, l'*IS*IIS, ne sont pas des instruments conçus pour observer les organismes plus petits que 600 µm (l'UVP peut certes capturer des particules <600 µm mais la résolution n'est pas suffisante pour l'identification taxinomique). Or, c'est dans cette gamme de taille (micro- et meso-plancton) que se trouve une grande partie des Rhizaria (ex : Acanthaires, Phaeodaires, Nassellaires, etc.), certains parfois très abondants (ex : Acanthaires ; Michaels et al., 1995). Il convient donc d'utiliser d'autres systèmes d'imagerie *in situ*, telle que l'imagerie holographique *in situ* (Benfield et al., 2007) afin d'étudier ce composant microplanctonique des Rhizaria.

Au cours des analyses réalisées sur le jeu de données d'images acquises par l'UVP, nos efforts se sont concentrés sur l'identification des différentes catégories de Rhizaria puis l'estimation de leurs abondances et biomasses (Chapitre III). Or, chaque image enregistrée par

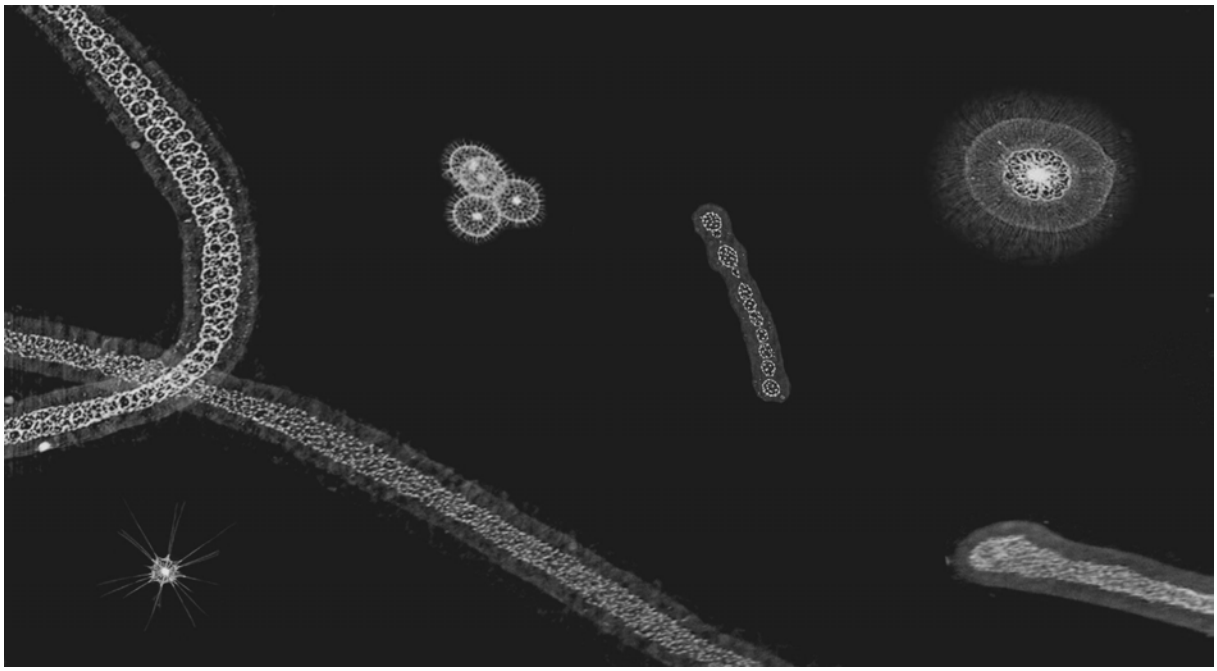


Figure 23 | Illustration de différents Rhizaria observés grâce à l'*IS*IIS (*In Situ* Ichtyoplankton Imaging System. Images issues du projet de science participative Plankton Portal (http://www.planktonportal.org)

l'UVP est géo-localisée dans le temps et dans l'espace, mais aussi dans la niche écologique qu'elle occupe, car associée à de nombreux paramètres physico-chimique relevés par les différents capteurs associés à l'UVP (ex : sonde CTD, capteur de nitrate ISUS, etc.). De ce fait, comme pour les analyses de diversité des *barcodes* environnementaux, et pour faire suite aux travaux présentés dans le Chapitre III, j'envisage des analyses de corrélations appliquées à ces données, ce qui rajouterait une dimension quantitative à l'étude de l'écologie des Collodaires. De plus, grâce à la couverture géographique globale et à l'hétérogénéité des écosystèmes rencontrés, la détermination des niches écologiques n'en sera que plus résolutive. Ces travaux d'études de l'écologie des Rhizaria, et notamment ceux des Collodaires, pourront également aborder les possibles interactions biotiques entre les différents composants des communautés planctoniques observés par ailleurs avec l'UVP. Enfin, des études récentes suggèrent que les écosystèmes oligotrophiques seront fortement influencés par les changements climatiques (Behrenfeld et al., 2006 ; Polovina et al., 2008). Au vu de la distribution préférentielle des Collodaires dans ces écosystèmes (Chapitre III), il serait intéressant de tester expérimentalement, ou de modéliser, si ces changements à grande échelle peuvent influencer la distribution des Collodaires, comme cela semble être le cas pour d'autres groupes planctoniques tels que les copépodes (Helaout et Beaugrand, 2007 ; Reygondeau et Beaugrand, 2011).

3.2. Du *barcode* à l'environnement

a. Biodiversité des Collodaires

L'utilisation des outils moléculaires a permis de révolutionner les études de biodiversité tout en s'affranchissant des identifications morphologiques, particulièrement laborieuses (Díez et al., 2001 ; López-García et al., 2001 ; Moon-van der Staay et al., 2001). Grâce à la sélection d'un grand nombre d'échantillons acquis lors de l'expédition *Tara* Océans, la couverture géographique de notre étude a permis d'examiner les patrons de biodiversité des Collodaires à travers une large gamme d'écosystèmes (ex : upwelling, gyres et fronts océaniques, etc.). Cette biogéographie (Chapitre II-1) a notamment confirmé une des hypothèses émises auparavant, stipulant que les Collodaires sont des organismes en grande majorité ubiquitaires (Strelkov et Reshetnyak, 1971 ; Swanberg, 1979). Même si certaines familles semblent occuper des niches écologiques préférentielles (ex : Collophidiidae en profondeur), les Collodaires, dans leur ensemble, semblent s'être adaptés à de nombreuses conditions environnementales. Néanmoins, ces conclusions sont principalement dictées par les résultats obtenus lors de l'étude phylogéographique mettant en jeu la région V9 du gène ribosomal 18S. Or, comme cela a été évoqué précédemment (sous-partie 1.1), ce court fragment d'ADN n'offre qu'une faible résolution taxinomique (Figure 20). Ainsi, l'interprétation de la distribution de la biodiversité des Collodaires est donc principalement basée sur la distribution des clades et non des différentes espèces. Afin de répondre à ces contraintes, l'utilisation d'autre *barcodes* pourrait améliorer la résolution taxinomique et permettre l'étude détaillée de la distribution des différentes espèces de Collodaires. Une telle problématique n'est pas restreinte à la seule étude de la biogéographie des Collodaires. Chez la cyanobactérie marine du genre *Synechococcus*, le gène *petB* (gène codant pour la sous-unité b6 du cytochrome) s'est

ainsi révélé beaucoup plus efficace que le gène 16S rRNA, traditionnellement utilisé, pour discriminer la distribution géographique des différents écotypes de cette cyanobactérie marine (Mazard et al., 2012).

b. Quelles limites pour le *metabarcoding* ?

Une partie des travaux de cette thèse s'est inscrite dans un contexte récent où l'utilisation des techniques d'exploration moléculaire a exposé la grande diversité et l'abondance des Collodaires dans les océans (Countway et al., 2007 ; Not et al., 2007 ; de Vargas et al., 2015 ; Pernice et al., 2015). Néanmoins, les travaux réalisés au cours de cette thèse, et notamment ceux portant sur la diversité environnementale des Collodaires (Chapitre II-1), sont venus apporter un regard nouveau sur leur biodiversité grâce aux bénéfices de la PCR quantitative (qPCR) qui a nous permis de choisir un seuil d'abondance spécifique leur étant spécifique. Un tel seuil, associé à un seuil d'identité génétique spécifique aux Collodaires et préalablement déterminé, nous a permis d'optimiser le regroupement des différents *métabarcodes* en entités génétiques similaires. Parmi les 230 unités taxonomiques opérationnelles (OTUs) définies dans l'ensemble du jeu de données issu de l'Expédition *Tara* Océans, une dizaine d'OTUs n'ont pu être observés que dans quelques stations d'échantillonnage (Chapitre II-1, Figure 4b). Même si l'on ne peut pas exclure la présence d'espèces endémiques et/ou cryptiques, il a récemment été démontré que la variabilité intra-organisme (c'est-à-dire au sein du génome d'une même cellule) pouvait être importante chez les Rhizaria et potentiellement mener à une surestimation de la diversité estimée par le *metabarcoding* (Pillet et al., 2012 ; Decelle et al., 2014). Afin d'estimer l'importance de la variabilité intra-génomique chez les Collodaires, des analyses préliminaires ont été effectuées sur 1) des librairies de clones construites à partir de plusieurs spécimens de Collodaires (Table 1) ; et 2) des données de séquences haut-débit générées pour une colonie. Même si ces jeux de données n'ont pas encore été complétement analysés en détail, les résultats obtenus semblent indiquer qu'au sein d'un Collodaire (solitaire ou colonie) la variabilité intra-individuelle (exprimée ici par le nombre de sites polymorphiques d'un fragment de ~1200 paires de bases du gène ribosomal 18S) varie entre 1,2% et 11% (Table 1). Ainsi, au sein même d'une colonie, l'utilisation de séquençage haut-débit pourrait conduire à la production d'une multitude de séquences distinctes ne reflétant que la variabilité intra-individuelle, biaisant ainsi toute estimation de diversité. L'analyse détaillée de ces deux jeux de données (clonage et séquençages haut-débit) devrait pouvoir nous en apprendre davantage sur les potentielles limites de l'utilisation du gène ribosomal

Tableau 1 | Pourcentage de différences et de sites polymorphiques du gène ribosomal 18S chez des Collodaires solitaires et coloniaux. (π) Diversité nucléotidique ; (Hd) Diversité haplotypique.

ID	Form	Clones (n)	Unique sequences (n)	Length (bp)	Pairwise Difference (%)			Polymorphic Sites (%)	Hd	π (x 10^{-2})
					Mean	Max	S.D.			
Pac1	Sol	23	17	1206	0.34	0.83	0.07	31 (2.6)	0.972	0.348
Pac7	Col	15	8	1184	0.22	0.76	0.07	14 (1.2)	0.886	0.217
Pac9	Col	48	36	1205	0.34	1.00	0.07	53 (4.4)	0.985	0.35
Pac15	Sol	26	20	1204	2.87	9.38	0.26	125 (10.4)	0.975	2.88
Pac19	Col	20	19	1204	2.36	9.30	0.22	132 (11)	0.995	2.375

18S dans les approches de *metabarcoding* pour les Collodaires, et devrait nous permettre de définir des entités génétiques robustes.

c. Vers un *metabarcoding* quantitatif ?

L'approche de *metabarcoding* utilisée pour l'analyse de la diversité environnementale des Collodaires (Chapitre II) nous a permis d'étudier la distribution géographique des différents clades identifiés lors notre révision de leur classification (Chapitre I). Cependant, cette approche ne nous a pas permis de quantifier l'abondance absolue des Collodaires à partir de leur nombre de copies du gène ribosomal 18S. Même si l'effort a été fait de mesurer le nombre total de copies au sein d'un seul Collodaire, les résultats trop variables entre les deux formes de Collodaires ne nous permettent pas de convertir ce nombre de copies, extraites de l'environnement, en nombre de capsules centrales.

Cependant, étant donné la corrélation positive entre le nombre de copies du gène 18S et la taille ou le biovolume des organismes (Zhu et al., 2005 ; Godhe et al., 2008), certaines études ont tenté une comparaison entre les abondances de *barcodes* et les abondances d'organismes estimées en microscopie optique (ex : de Vargas et al., 2015). Malgré cette corrélation, les *barcodes* n'apparaissent pas systématiquement comme étant de très bons *proxy* pour déterminer le biovolume des organismes, et encore moins leurs abondances cellulaires. Dans le cas de groupes spécifiques tels que les diatomées, la comparaison des abondances des différents genres estimées par microscopie avec les abondances relatives de *barcodes* fournit quelques résultats cohérents (de Vargas et al., 2015). Néanmoins, pour les Collodaires, une telle comparaison entre comptages microscopiques et abondances relatives de *barcodes* semble impossible au vu des dommages infligés aux Collodaires par les filets à plancton, qui nuiraient à cette comparaison.

Cependant, étant donné la collecte réalisée en parallèle avec les filets à plancton et l'UVP au cours de certaines stations d'échantillonnages de l'expédition *Tara* Océans, nous avons tenté d'établir une comparaison entre les abondances relatives de *barcodes* avec les estimations quantitatives enregistrées par l'UVP. Pour cela, nous avons estimé le nombre de *barcodes* que représenteraient les organismes observés par l'UVP en utilisant les valeurs moyennes enregistrées en PCR quantitative (Chapitre II-1 – Figure 1). Même si ces deux types de prélèvements n'ont pas été réalisés de façon simultanée et consistent en des prélèvements obliques (filets) et verticaux (UVP), nous avons essayé de maximiser la comparaison en sélectionnant uniquement les images enregistrées par l'UVP entre la surface et la DCM. De plus, ces instruments ne couvrant pas la même gamme de taille, nous n'avons sélectionné que les échantillons de *metabarcoding* pour la fraction 180 – 2 000 µm, et mis un seuil de taille entre 600 – 2 000 µm pour l'UVP, afin de se rapprocher d'une gamme analogue. Pour 49 stations de prélèvements les *barcodes* n'ont pu être comparés aux images à cause de l'absence de ces dernières. Sur les 46 stations où les abondances de copies ont pu être comparées, il apparaît que l'UVP sous-estime le nombre de copies d'ADN en comparaison du nombre estimé *via* le *metabarcoding* (Figure 24). Malgré les efforts mis en jeu pour sélectionner une gamme de taille et des profondeurs d'échantillonnage comparables, d'autres paramètres semblent biaiser la comparaison. Ainsi, les résultats présentés ici ne

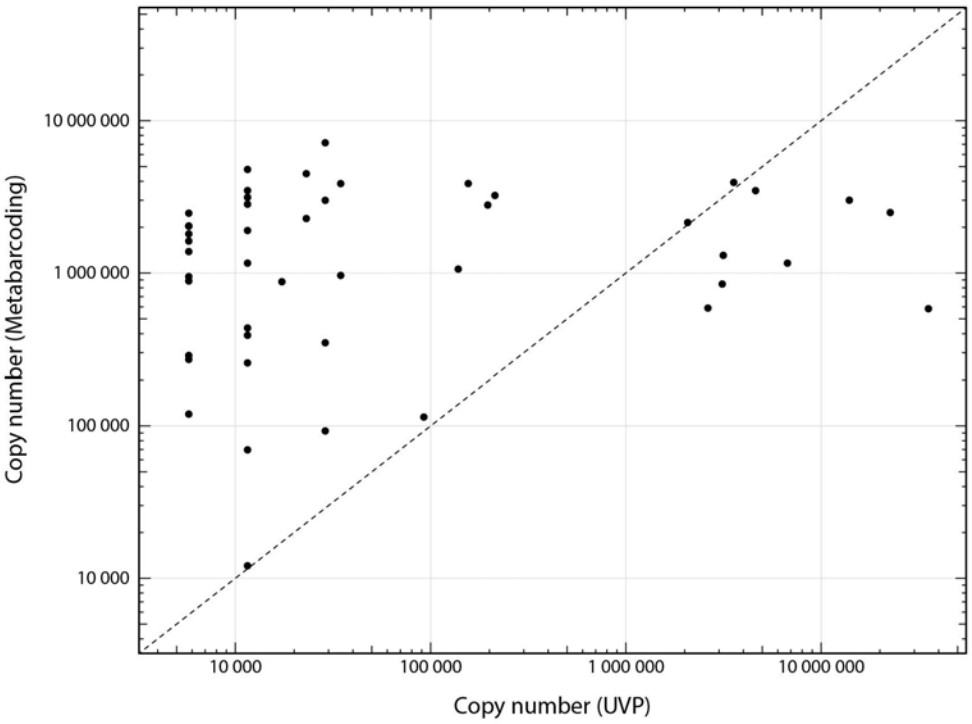


Figure 24 | Comparaison du nombre de copies d'ADN estimée à partir des images UVP et celle enregistrée grâce au *metabarcoding*. La courbe en pointillé montre une relation 1:1.

prennent pas en compte les volumes échantillonnés par les deux méthodes de prélèvements, qui pourtant diffèrent presque d'un facteur 100 : ~1.7 m^3 pour un profil vertical d'UVP de 0 – 100 m ; ~200 m^3 pour deux traits horizontaux (surface et DCM) avec un filet Bongo. Il convient donc de prendre en compte les volumes échantillonnés de chaque profil UVP et traits de filets, afin d'accéder à une comparaison plus robuste.

Malgré les difficultés apparentes à considérer les *barcodes* comme des *proxy* quantitatifs fiables, la comparaison des méthodes moléculaires avec des méthodes d'imagerie n'est pas à exclure dans un futur proche. Il y a peu, l'utilisation de la plateforme d'échantillonnage HRS (High Resolution Sampler), où un compteur de particules (OPC), un système d'imagerie *in situ* (SIPPER) et des filets à plancton étaient mis en série, a permis une comparaison directe entre les différents outils d'échantillonnage (Remsen et al., 2004). L'utilisation d'une telle plateforme d'échantillonnage pour l'étude des Collodaires pourrait permettre cette comparaison directe entre nombre de copies (estimé dans les échantillons collectés par le filet) et les abondances estimées par le système d'imagerie. Néanmoins, il semble difficile de pouvoir déployer en routine de telles plateformes encombrantes et nécessitant une logistique conséquente. En revanche, l'utilisation de caméras *in situ* miniaturisées, montées sur le cadre des filets à plancton, permettrait de visualiser directement le plancton collecté par le filet et offrirait une comparaison directe entre le contenu du collecteur et les images *in situ*. La caméra devra être idéalement placée à quelques centimètres en retrait de l'ouverture pour 1) observer les organismes intacts, et 2) éviter de capturer des organismes refoulés par la suite en dehors du filet. Même si de tels systèmes hydrides de collecte ont déjà vu le jour (Olney et Houde, 1993 ; Schulz et al., 2010), leur utilisation n'a jamais été globale. Pourtant, grâce aux avancées technologiques actuelles en matière d'imagerie *in situ*, ainsi qu'à l'amélioration des

performances de classification taxonomique semi-automatique (ex : utilisation de réseaux de neurones - http://benanne.github.io/2015/03/17/plankton.html) associée à des initiatives de sciences participatives (ex : Plankton Portal - http://www.planktonportal.org), le développement de ces méthodes alternatives d'échantillonnage semble prometteur. Enfin, au-delà du cas spécifique des Collodaires, l'utilisation de telles plateformes d'échantillonnage ou de systèmes hybrides pourrait offrir de nouvelles perspectives dans l'étude de la diversité et l'écologie du plancton fragile.

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ANNEXES

Annexe 1 :
Présentations orales et posters

Annexe 2 :
Campagnes en mer

Annexe 3 :
Travail en collaboration

Annexe 1 : Présentations orales et posters

Communications orales :

Monod Conference, Roscoff, France (June 2015) - *Rhizaria, the elusive stars of the ocean.*

INTERRAD, Antalya, Turkey (March 2015) - *Morpho-molecular classification of the Collodaria: Toward an integrative taxonomy?* ***Travel Grant*** *- € 290*

Aquatic Sciences Meeting, Granada, Spain (February 2015) - *Rhizaria: elusive stars of the ocean.*

Sixth Young Researchers Day, Roscoff, France (December 2014) - *Rhizaria: elusive stars of the ocean.* ***3RD Prize*** *of the best presentation.*

Fifth Young Researchers Day, Roscoff, France (December 2013) - *Molecular diversity of Collodaria (Radiolaria) gives insight on their life cycle.*

Posters :

INTERRAD, Antalya, Turkey (March 2015) - *Rhizaria (Radiolaria and Phaeodarea), the elusive stars of the ocean.*

2ème Colloque de Génomique Environnementale, Rennes, France (November 2013) - *Molecular diversity of Collodaria (Radiolaria) gives insight on their life cycle.*

Fourth Young Researchers Day, Roscoff, France (December 2012) - *Diversity and Ecological Significance of Radiolarian Symbiosis.*

Sciences participatives (Projet collaboratif avec le Réseau de Sciences Marines Participatives) :

Les Rencontres du RIEM, Lorient, France (Mars 2014) - *Ethanol Fix Protocol (Belle-île), Bilan.*

Les Saventuriers de la Mer, Lorient, France (Mars 2013) - *Eco-exploration marine participative.*

Annexe 2 : Campagnes en mer

Hokkaido University cruise aboard *Oshoro-Maru* (August 2013)

SCRIPPS Institution of Oceanography cruise CCE-LTER P1408 Process Cruise aboard *R/V Melville*

Cruise report from SBR group activities (August-September 2014)

During this cruise we have focused on the distribution and community structure of phaeodarian, radiolarian, and free-living symbiotic microalgae species across the different water masses (coastal-to-offshore) of the CCE system, their vertical structure in the water column and contribution to vertical export. In order to do so we have adopted a morpho-molecular approach that combines molecular and image analytical tools. We took samples for metabarcoding analysis of the DNA community composition using the 18S rRNA. We took samples for fluorescence in situ hybridization (FISH) analysis specific to a number of free-living symbiotic microalgae species (known to be symbionts of Radiolaria) for which we have fluorescence molecular probes designed. We also sorted out single cells in ethanol for single-cell image and molecular identification and preserved samples in ethanol for later molecular analysis in the lab. Phaeodarians and radiolarians encompass organisms with size that spans more than 3 orders of magnitude, with Acantharia ranging from 50-1000 µm diameter, larger phaeodarian cells on the millimeter range, and larger colonies of various centimeters long. We have combined different sampling approaches including net tows and CTD niskin bottles cast in order to cover this wide range of sizes and concentrations in the water.

1. We have used two late evening CTD cast per cycle sampling 6 depths. We did two CTD's during Cycles 1, 2 and 3; and one CTD during cycles 4 and 5. We collected the following samples for each depth:

- -One entire niskin bottle (10 L) was concentrated into 250 mL using 5 µm mesh net, filtered onto 8 µm supor membrane filter and flash freeze for DNA community analysis (n=48 samples).
- -3 L filtered onto 0.8 µm support filter for DNA community analysis of the nano- size fraction (n=48).
- -1 L filtered onto 0.2 µm support filter for DNA community analysis of the pico- size fraction (n=48).
- -0.25 L filtered onto 3 µm polycarbonate filters for FISH analysis of the free-living symbiotic microalgae (only one CTD per Cycle, n=30 samples).
- -0.3 L filtered on 3 µm polycarbonate filters for scanning microscopy analysis. The main purpose of these particular samples is to analyze the thickness and calcification state of the coccolithophorids across oxygen gradients (only one CTD per Cycle, n=30 samples).
- -15-25 L were concentrated into 250 mL using phytoplankton 5 µm mesh net, 50 mL were preserved in ethanol for molecular analysis, and the other 200 mL fixed with formalin and complemented with $SrCl_2$ for microscopy quantitative analysis back in the lab (n=48 ethanol and 48 formalin + $SrCl_2$ samples).

2. We took a subsample of the day bongo net tow daily. We got $1/8^{th}$ split sub-sample of the net tow, which was further split into:

- $1/16^{th}$ split was filtered on 100 µm polycarbonate filter and store in LN2 for molecular analysis of radiolarian community.
- $1/16^{th}$ part was fixed with pure ethanol.

3. We did a ring net tow (330 µm mesh size) per cycle to sort single specimens of interests, image them for morphologic identification and preserved them in ethanol for single-cell molecular ID and

scanning microscopy of skeleton-bearing individuals. This ring net aimed at collecting enough live radiolarians to conduct in situ incubations in the array. During Cycles 1,2, and 3 we focused on phaeodarians and collodarians (radiolarian group forming large symbiotic colonies or large solitary forms). We sorted out several phaeodarians morphotypes including those most frequently associated to the thick layer of Radiolaria found below the euphotic zone in previous CCE cruises. Molecular ID of these specimens will be performed back in the lab. Partly due to the abundance of jelly organisms (salps mainly) no specimens were recovered in good shape for conducting incubations. During Cycles 4 and 5 we found a very different community in our ring net tows, which free of salps tyranny allowed us to sort out specimens in better shape for incubating in the array. We performed two types of experiments:

- On one hand we assessed the concentration of dimethyl sulfur compounds in symbiotic radiolarians incubated across 4 depths in the water column. The objective of these experiments is to test the putative antioxidant role of these compounds in phytoplankton and symbiotic species in particular. We hypothesis that DMSP concentration will decrease under higher irradiance conditions of shallower incubations due to its capacity to scavenge hydroxyl radicals and form DMSO.
- On the other hand we conducted in situ 14C and 15N incubations to assess primary productivity from two different angles at surface and deep chlorophyll maximum depths. We also measured chlorophyll per colony biovolume, which gave measurable, and for some species surprisingly high values. For instance, one solitary symbiotic collodarian specimen, yielded similar chlorophyll values to approximately 50 mL of DCM water in the same stations. We performed a total of 3 DMSP and 4 productivity experiments (with colonies and solitary collodarians).

4. To assess the contribution of phaeodarians and radiolarians living in the first 100-150 m of the water column to vertical fluxes we plugged into the sediment traps deployments leaded by Mike Stukel. We followed the same morpho-molecular approach we adopted for the water column. We collected samples from two tubes at each depth of deployment in each of the cycles. One of the tubes fixed with formalin while the other was deployed without preservatives to facilitate the DNA extraction and following up analysis. This in itself will offer an interesting methodological comparison to assess the influence of the degradation or consumption rates on the community composition recovered after 3 days of deployment. We quantitatively split the content of each of the tubes into different filters (0.2, 0.8, 8 μm pore size), formalin + $SrCl_2$ fixation and ethanol preservation for molecular and microscopy analysis. We also took samples for pigment analysis at each depth from both the formalin preserved and non-preserved tubes.

5. We used opportunistic deep casts during the cruise to sample the community of radiolarians that has been reported to inhabit deep waters (700-3000 m), but from which we know very little about. We focused on the DNA community larger than 5 microns by filtering 20-40 L of water from 700 m in Cycles 3 and 4 (previous concentration with >5 μm net) and 20 L at 1000, 2000, and 3000 at cycle 5. Our main focus is on the radiolarians and phaeodarians. However, if questions regarding other groups and taxa arise during the analysis, we will be able to dig into the DNA samples as well as the formalin fixed or ethanol preserved samples. We also conducted daily mini dilution experiments in the array during the cruise. These experiments belong for the most part to a different topic and objectives to those related to radiolarians, although part of them are linked. They are designed to assess the response of phytoplankton growth and grazing mortality under different in situ irradiance conditions. Water was collected from a single depth below the mixed layer where photosynthesis was expected to be light-limited, six replicated mini dilution experiments were prepared and incubated across the 6 depths of the array. Two additional mini dilution experiments were systematically prepared; one

incubated in the dark at the shallowest depth, and one extra experiment incubated at the depth of water collection to improve the accuracy of the rate estimates at original depth of collection and to get a sense of the reproducibility of the experiments. This design encompassed a total of 8 mini dilution experiments per array. Samples for flow cytometry were taken in every experiment. Samples for DNA at 0.45 µm were taken in the first two days of each cycle for qPCR analysis of specific groups of picophytoplankton (e.g. picocyanobacterial clades), while samples for FISH analysis were taken only in the third day of experiments in each cycle. These FISH sampling pursue to assess the growth and grazing mortality rates of free-living symbionts of Radiolaria like *Phaeocystis* sp. (Prymnesiophyceae, symbiont of Acantharia) and *Brandtodinium* (Dinophyceae, symbiont of polycystines radiolarians).

6. We actively collaborate with Marc Picheral during this cruise to focus on the distribution along the water masses of the different rhizarian groups (mainly phaeodarians and collodarians) using the UVP. For each CTD profile, we sorted the different rhizarian and were able to infer their abundances as well as their contribution to overall zooplankton community (>1 mm). From previous CCE cruises, a category of organisms was associated with the phaeodarian. This category showed high abundances and biomasses (especially between 125-150 m), sometime locally higher than copepods. To assess the validity of our identification as a phaeodarian, we isolated a set of different rhizarian organisms and calibrated the UVP on board. The UVP was removed from the CTD rosette, placed into a seawater container, and the different organisms dropped one by one in the imaged volume. Optical microscopic observations were then compared to the "*in-situ*" images provided by the UVP. We therefore confirmed that these highly abundant organisms previously observed, belong to the phaeodarian. Molecular single-cell barcoding and morphological ID should later provide a name to this organism.

Annexe 3 : Travail en collaboration

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BRANDTODINIUM GEN. NOV. AND *B. NUTRICULA* COMB. NOV. (DINOPHYCEAE), A DINOFLAGELLATE COMMONLY FOUND IN SYMBIOSIS WITH POLYCYSTINE RADIOLARIANS[1]

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Symbiotic interactions between pelagic hosts and microalgae have received little attention, although they are widespread in the photic layer of the world ocean, where they play a fundamental role in the ecology of the planktonic ecosystem. Polycystine radiolarians (including the orders Spumellaria, Collodaria and Nassellaria) are planktonic heterotrophic protists that are widely distributed and often abundant in the ocean. Many polycystines host symbiotic microalgae within their cytoplasm, mostly thought to be the dinoflagellate *Scrippsiella nutricula*, a species originally described by Karl Brandt in the late nineteenth century as *Zooxanthella nutricula*. The free-living stage of this dinoflagellate has never been characterized in terms of morphology and thecal plate tabulation. We examined morphological characters and sequenced conservative ribosomal markers of clonal cultures of the free-living stage of symbiotic dinoflagellates isolated from radiolarian hosts from the three polycystine orders. In addition, we sequenced symbiont genes directly from several polycystine-symbiont holobiont specimens from different oceanic regions. Thecal plate arrangement of the free-living stage does not match that of *Scrippsiella* or related genera, and LSU and SSU rDNA-based molecular phylogenies place these symbionts in a distinct clade within the Peridiniales. Both phylogenetic analyses and the comparison of morphological features of culture strains with those reported for other closely related species support the erection of a new genus that we name *Brandtodinium* gen. nov. and the recombination of *S. nutricula* as *B. nutricula* comb. nov.

Key index words: **dinoflagellate; Peridiniales; polycystines; Radiolaria; *Scrippsiella*; symbiosis; taxonomy; *Zooxanthella***

***Abbreviations*: ICBN, International Code for Botanical Nomenclature; ITS, internal transcribed spacer; LM, light microscopy; LSU, large subunit (ribosomal DNA); ML, maximum likelihood; PCR, polymerase chain reaction; RCC, roscoff culture collection; SEM, scanning electron microscopy; SSU, small subunit (ribosomal DNA)**

Mutualistic associations involving photosynthetic microalgae are common in both benthic and pelagic ecosystems and are essential for establishing and maintaining the structure of marine communities (Caron 2000). Symbiosis between corals and the dinoflagellate genus *Symbiodinium* Freudenthal is fundamental for the survival and ecological success of coral reef ecosystems. Members of the genus *Symbiodinium* have been intensively studied with respect to their morphology and life cycle (Freudenthal 1962, Fitt and Trench 1983, Trench and Blank 1987), and genetic diversity (Coffroth and Santos 2005, Sampayo et al. 2009, LaJeunesse and Thornhill 2011, Stat et al. 2011). Studies on this coastal benthic symbiotic relationship significantly increased

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when the coral-bleaching phenomenon was brought to global attention and associated to increases in sea surface temperature, enhanced light intensity, and ocean acidification (Hoegh-Guldberg et al. 2007).

Symbiotic interactions between pelagic hosts and microalgae have received less attention, although they are widespread in the photic layer of the world ocean where they play a fundamental role in the ecology of the planktonic ecosystem (Stoecker et al. 2009, Decelle et al. 2012a,b). Recent studies have demonstrated that dinoflagellate symbionts of Foraminifera belong to *Pelagodinium* Siano, Montresor, Probert et de Vargas, a genus that is related to *Symbiodinium* within the order Suessiales (Siano et al. 2010), and that Acantharia typically associate with members of the prymnesiophyte genus *Phaeocystis* Lagerheim (Decelle et al. 2012a,b), although one taxon, *Acanthochiasma* sp., can contain multiple symbiotic partners, including distantly related dinoflagellates (from the genera *Pelagodinium*, *Heterocapsa* Stein, *Azadinium* Elbrächter et Tillmann and *Scrippsiella* Balech ex Loeblich III) as well as a haptophyte (Decelle et al. 2012b).

Polycystine radiolarians (including the orders Spumellaria, Collodaria, and Nassellaria) are single-celled, heterotrophic, biomineralizing planktonic protists from the Rhizaria lineage that are widely distributed in the ocean and are found throughout the entire water column (Boltovskoy et al. 2010). Many polycystines host microalgae within their cytoplasm (Anderson 1983). Cells containing photosynthetic microalgae have been shown to survive for longer periods in nutrient-poor water than those that do not have microalgal partners and the microalgae are therefore assumed to be symbionts that play a nutritive role for the hosts (Anderson 1983).

Polycystines form associations with various dinoflagellate, prymnesiophyte and prasinophyte partners (usually not at the same time), with dinoflagellates being the most common symbiotic partners (Anderson 1976, 1983, Anderson et al. 1983). In the late nineteenth century, Karl Brandt was the first to recognize that the "yellow cells" within polycystines, actinian corals and hydrozoans were microalgae, which he collectively described in the new genus *Zooxanthella* Brandt (Brandt 1881), although they were not immediately recognized as dinoflagellates. Soon afterward, the species *Z. nutricula* Brandt was proposed for the symbiont of the collodarian polycystine *Collozoum inerme* collected from the western Mediterranean Sea and it was stated in the description that this species was presumably identical to the yellow cells of other polycystines (Brandt 1882). The subsequent taxonomic history of this genus and species have been very confused (see review by Blank and Trench 1986), and the plural noun "zooxanthellae" has persisted as a colloquialism used to describe marine microalgal endosymbionts in general.

The symbionts of the "by-the-wind sailor" hydrozoan jellyfish *Velella velella* were reported to be similar to those of polycystines by Hovasse (1922), who initially described the in hospite symbionts of Mediterranean *V. velella* as *Endodinium chattoni* Hovasse (*E. chattonii* under International Code for Botanical Nomenclature (ICBN) Art. 73). Taylor (1971) and Hollande and Carré (1974) further characterized the *in hospite* stage of *E. chattonii* and the latter authors proposed the reclassification of the polycystine symbionts (*Z. nutricula*) as *E. nutricola* (Brandt) Hollande et Carré (*E. nutricula* under ICBN Art. 73), although Hovasse (1924) had in fact previously recombined *E. chattonii* as *Z. chattonii* (Hovasse) Hovasse. Banaszak et al. (1993) isolated a culture of the symbiont of *V. velella* from the Pacific, which they considered slightly different from *E. chattonii* (larger cell size and presence of trichocysts in hospite and in culture). Based on scanning electron microscopy (SEM) observations of the morphology and arrangement of thecal plates in the motile stage, Banaszak et al. (1993) classified their organism in the genus *Scrippsiella* as a new species, *S. velellae* Banaszak, Iglesias-Prieto et Trench (a name later validated by Trench 2000). These authors also transferred *E. chattonii* and *E. nutricula* to *Scrippsiella* as *S. chattonii* (Hovasse) Banaszak, Iglesias-Prieto et Trench, and *S. nutricula* (Brandt) Banaszak, Iglesias-Prieto et Trench, respectively (Banaszak et al. 1993), but these names remain technically invalid because reference was not made to the exact page of the basionym.

Using molecular methods, Gast and Caron (1996) found that the dinoflagellate symbionts in six different polycystine species from the Sargasso Sea (the collodarians *Collozoum caudatum* and *Thalassicolla nucleata*, three unidentified collodarian species and the spumellarian *Spongostaurus* sp.) had identical SSU rDNA sequences that they assigned to *S. nutricula*. These molecular analyses indicate that taxonomically divergent radiolarians can contain the same symbiotic dinoflagellate. Since these analyses were conducted directly on symbionts extracted from the hosts (i.e., not cultured), the morphology of the motile stage of the symbiotic algae assigned to *S. nutricula* was not investigated, and has still never been reported. Gast and Caron (1996) also sequenced the SSU rDNA of the symbiont of *V. velella* from the Sargasso Sea and found that the sequence was very similar to those of the radiolarian symbionts (four differences out of 1,802 base pairs). They therefore also assigned this *V. velella* symbiont to *S. nutricula*.

Here, we examined the morphology and molecular phylogenetic position of clonal cultures of the free-living stage of dinoflagellates isolated from several different polycystine radiolarian hosts, including *Collozoum*, the taxon from which *Z. nutricula* was originally described. In addition, we sequenced symbiont genes directly from several polycystine-symbiont

holobiont specimens (including collodarian, spumellarian and nassellarian hosts) from different oceanic regions. Accurate morpho-molecular characterization and taxonomic designation of symbionts from the genus *Symbiodinium* has been key for studies of the ecology and functioning of coral reef systems and it is likewise likely to prove important for future studies on the widespread pelagic symbiosis involving polycystine radiolarian hosts.

MATERIAL AND METHODS

Samples and culture isolation. The radiolarian specimens from which the holobiont sequences or cultures originated were isolated from samples collected in 2010–2012 by net tows (20–150 μm mesh size) in the bay of Villefranche-sur-Mer (France), off Sesoko Island, Okinawa (Japan) and in the South Pacific Ocean during the Tara Oceans expedition (Table 1; Figs. S1 and S2 in the Supporting Information). The polycystines were first sorted from fresh net samples under a binocular microscope, cleaned by successive transfers in sterile seawater in petri dishes, and then left in an illuminated and temperature-regulated incubator for several hours to self-clean. Individual clean specimens were then identified based on their morphology and imaged under an inverted microscope. Some specimens were then transferred to guanidinium isothiocyanate buffer for direct DNA extraction from holobionts. The dinoflagellate cultures were obtained by micropipette isolation of single symbiont cells released from live radiolarian specimens that were microdissected under an inverted microscope. The resulting monoclonal cultures were maintained in filter-sterilized seawater with K/2(-Tris, -Si) medium supplements (Keller et al. 1987) at 22°C with an irradiance of 70–80 μmol photons $\cdot m^{-2} \cdot s^{-1}$ in a 12:12 light:dark regime. The cultures have been deposited in the Roscoff Culture Collection (RCC; http://www.roscoff-culture–collection.org). Light microscopy (LM) images of radiolarian holobionts from which sequences/cultures were obtained are shown in Figures S1 and S2. Detailed information related to each of the samples used in this study can be found in the RENKAN database at http://abims.sb-roscoff.fr/renkan/.

Microscopy preparations and observations. Light micrographs of living cells were taken using a Zeiss Axiophot light microscope equipped with a Zeiss AxioCam digital camera system (Carl Zeiss, Oberkochen, Germany). For SEM, dinoflagellate cells were fixed in 1% (v:v) formol for 2 h at room temperature. Samples were then gently filtered onto 3 μm pore-size Nucleopore polycarbonate filters (Pleasanton, CA, USA), washed with distilled water, dehydrated in an ethanol series (25%, 50%, 75%, 95%, 100%), and critical-point-dried. The filters were mounted on stubs, sputter coated with gold, and examined with a FEI Quanta™ 200 SEM (FEI, Hillsboro, OR, USA).

DNA extraction, sequencing, and phylogenetic analyses. Genomic DNA was extracted from exponentially growing cultures of the strains using a NucleoSpin Plant II DNA extraction kit (Macherey-Nagel), or from holobionts using the method described in De Vargas et al. (2002).

Partial nuclear large subunit (LSU) and small subunit (SSU) rDNA genes were polymerase chain reaction (PCR) amplified using Phusion high-fidelity DNA polymerase (Finnzymes, Vantaa, Finland) in a 25 μL reaction volume and the following thermocycler steps: an initial denaturation step at 98°C for 30 s, followed by 35 cycles at 98°C for 10 s, 30 s at the temperature of semi-hybridization chosen for each set of primers, and 30 s at 72°C, with a final elongation step of 10 min at 72°C. The eukaryote primer set 63F (ACGCTT GTCTCAAAGATT)/1818R (ACGGAAACCTTGTTACGA; Tm 50°C; Lepere et al. 2011) was used to amplify the SSU rDNA of the dinoflagellate cultures, whereas the dinoflagellate specific primer set DIN464F (TAACAATACAGGGCATCCAT)/S69 (CCGTCADTTCCTTTRAGDTT; Tm 53°C) was used to target the dinoflagellates in the holobiont samples. The D1-D2 fragment of the LSU rDNA was amplified using the dinoflagellate specific primers Ldino6 (MCC CGCTGAATTTAAGCATA)/Ldino1 (AACGATTTGCAGGTCAGTACCGC; Tm 55°C) from both cultures and holobionts. PCR products were then sequenced at the GENOSCOPE (CEA, Evry, France).

The sequences generated from the studied strains and holobionts (GenBank accession numbers KF557491 to KF557545) were aligned with other LSU and SSU rDNA sequences from GenBank (release 194.0, February 2013) attributed to *Scrippsiella* and related Peridiniales genera, as well as representatives of the Suessiales as an outgroup. Alignments were generated using MUSCLE implemented in Seaview v.4.0 (Gouy et al. 2010) with subsequent manual verification. The LSU rDNA data set contained 48 sequences (675 unambiguously aligned positions) and the SSU rDNA data set contained 57 sequences (652 unambiguously aligned positions).

Phylogenetic analyses were conducted with maximum likelihood (ML) and Bayesian methods. The ML analysis was carried out using MEGA v. 5.1 (Tamura et al. 2011) with the general time reversible as the best model of nucleotide substitution and considering a gamma distribution with a proportion of invariable sites (I) set at 5 by default. Bootstrap supports for the tree were obtained after 1,000 replicates. Bayesian analyses were conducted using Mr Bayes v.3.2.1 (Huelsenbeck and Ronquist 2001) using the same model of evolution. For each gene marker, two Markov chain Monte Carlo chains were run for 1 million generations, sampling every 500 generations (diagnostic frequency = 5,000). The standard deviation of split frequencies between the 2 runs was <0.01 in both LSU and SSU rDNA analyses. For both ML and Bayesian analyses, the trees were visualized and edited in Fig Tree v. 1.3.1 (Rambaut 2010). In the trees presented herein the posterior probabilities associated to each node in the Bayesian topologies are reported on the ML topologies.

RESULTS

Microscopy observations. In our culture conditions, the clonal strains of polycystine symbionts tended to contain a mixture of motile thecate cells and larger, irregularly shaped nonmotile cells devoid of the typical features of motile cells (theca, cingulum, sulcus), the latter more closely resembling the in hospite symbiotic state. The proportion of motile and nonmotile cells varied between strains and through growth cycles for each strain. The overall morphology and thecal plate pattern of motile cells was identical for several different strains observed. The following descriptions and illustrations are based on observations of strain RCC3387.

Cells are 10.5–15 μm in length (average 13.1 μm, $n = 30$) and 9.1–11.2 μm in width (average 10.4 μm, $n = 30$). The epitheca is larger than the hypotheca. Observed under LM, cells have a slightly convex conical epitheca with a well-pronounced apical horn (Fig. 1, A, B, D). The hypotheca is rounded (Fig. 1, A and D). The nucleus is large and occupies the center of the cells (Fig. 1, B and D). One or two

TABLE 1. List of specimens used to obtain symbiont sequences (images of host cells are shown in Figs. S1 and S2).

Host ID	Host taxonomy	Sampling site	Strain code	GenBank accession number	
				SSU rDNA	LSU rDNA
Holobionts					
PAC1	Collodaria (solitary)	South Pacific 21°17.462 S, 105°9.476 W	n.a.	KF557503	KF557534
PAC2	Collodaria (colony)	South Pacific 21°17.462 S, 105°9.476 W	n.a.	KF557504	n.a.
PAC3	Collodaria (solitary)	South Pacific 23°42.949 S, 107°20.141 W	n.a.	KF557505	KF557535
PAC4	Collodaria (solitary)	South Pacific 23°42.949 S, 107°20.141 W	n.a.	KF557506	KF557536
PAC6	Collodaria (solitary)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557507	n.a.
PAC7	Collodaria (colony)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557508	n.a.
PAC8	Collodaria (colony)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	n.a.	KF557537
PAC9	Collodaria (colony)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557509	KF557538
PAC10	Collodaria (solitary)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557510	n.a.
PAC11	Collodaria (solitary)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557511	KF557539
PAC14	Collodaria (solitary)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557512	KF557540
PAC15	Collodaria (solitary)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557513	n.a.
PAC16	Collodaria (colony)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557514	n.a.
PAC17	Collodaria (colony)	South Pacific 23°42.289 S, 131°12.744 W	n.a.	KF557515	KF557541
PAC19	Collodaria (colony)	South Pacific 23°42.289 S, 131°12.744 W	n.a.	KF557516	KF557542
PAC21	Collodaria (colony)	South Pacific 23°42.289 S, 131°12.744 W	n.a.	KF557517	KF557543
PAC22	Collodaria (colony)	South Pacific 23°42.289 S, 131°12.744 W	n.a.	KF557518	KF557544
PAC24	Collodaria (colony)	South Pacific 23°42.289 S, 131°12.744 W	n.a.	KF557519	n.a.
PAC26	Collodaria (colony)	South Pacific 23°42.289 S, 131°12.744 W	n.a.	KF557520	n.a.
PAC27	Collodaria (colony)	South Pacific 23°42.289 S, 131°12.744 W	n.a.	KF557521	n.a.
SES47	Collodaria (colony)	Sesoko, Japan 26°37′ 20 N, 127°52′ 15 E	n.a.	KF557502	KF557546
SES19	Spumellaria	Sesoko, Japan 26°37′ 20 N, 127°52′ 15 E	n.a.	KF557501	n.a.
SES28	Nassellaria	Sesoko, Japan 26°37′ 20 N, 127°52′ 15 E	n.a.	n.a.	KF557545
Vil 210	Spumellaria?	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	n.a.	KF557522	n.a.
Vil 217	Spumellaria	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	n.a.	KF557523	n.a.
Vil 219	Spumellaria	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	n.a.	KF557524	n.a.
Vil 231	Spumellaria	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	n.a.	KF557525	n.a.
Culture strains					
SES46	Collodaria (*Collozoum* colony)	Sesoko, Japan 26°37′ 20 N, 127°52′ 15 E	RCC3378 RCC3379	KF557500 KF557499	KF557526 n.a.
VFPO14	Collodaria (*Collozoum* colony)	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	RCC3380 RCC3381 RCC3382	KF557494 KF557495 KF557496	KF557530 KF557531 KF557532
VFPO2	Spumellaria	Villefranche-sur-Mer, France 43°41′ 10N, 7°18′ 50E	RCC3383 RCC3384	KF557491 n.a.	KF557527 KF557528

TABLE 1. Continued

Host ID	Host taxonomy	Sampling site	Strain code	GenBank accession number	
				SSU rDNA	LSU rDNA
VFPO5	Spumellaria	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	RCC3385	KF557492	n.a.
VFPO22	Spumellaria	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	RCC3386	KF557497	n.a.
VFR1	Spumellaria	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	RCC3387	KF557498	KF557533
VFPO10	Nassellaria	Villefranche-sur-Mer, France 43°41′ 10 N, 7°18′ 50 E	RCC3388	KF557493	KF557529

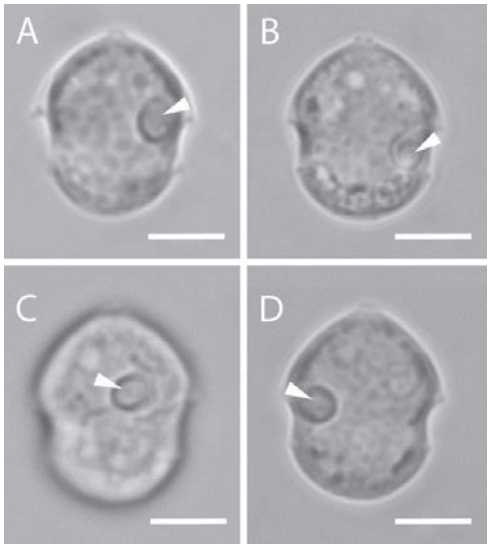

FIG. 1. Light micrographs of *Brandtodinium nutricula* gen. nov., comb. nov. (arrow indicates large pyrenoid). (A and B) Ventral view of the cell showing the large nucleus in the central portion of the cell (B). (C) Lateral (slightly apical) view of the cell. (D) Dorsal view of the cell; scale bars = 5 μm.

golden-yellow chloroplasts are present around the cell periphery, sometimes appearing as a single plastid bordering the cell periphery (Fig. 1D). One large circular pyrenoid (sometimes two) is often visible in LM (Fig. 1, A–D). No eyespot is visible in LM. Cells swim steadily in a straight line, rotating around the transapical axis. They suddenly stop, change direction at different angles from the original path, often back-tracking.

In SEM, the epitheca appears conical (Fig. 2A) to rounded (Fig. 2C), and the smaller hypotheca is symmetrical and rounded in ventral (Fig. 2A) and dorsal (Fig. 2C) view. The plate tabulation is Po, X, 4′, 3a, 7″, 5C, 4S, 5‴, 1⁗ (Fig. 2, A–E and Fig. 3, A–D). The pore plate (Po) is circular and surrounded by a high collar, and is connected to the first apical plate by a long well-defined rectangular canal plate (X; Fig. 2A, and Fig. 3, A and C). Three intercalary plates are interposed on the dorsal side of the cell between the apical series and the second epithecal (precingular) series (Fig. 2, C and D; Fig. 3, B and C). The first intercalary plate (1a) is five-sided and borders only one of the apical plates (2′), whereas the second and third intercalary plates (2a and 3a) are six-sided and both border two apical plates (Fig. 2, C and D; Fig. 3C). The cingulum is located in the median portion of the cell and descends slightly, displaced by approximately one-third of its own width (Fig. 2, A and C; Fig. 3, A and B). It is very wide and shallow and is constituted by a single series of five rectangular plates, the first being much narrower than the others (Fig. 2, A–C, E; Fig. 3, A and B). The sulcus is fairly shallow and narrows toward the antapical end (Fig. 2, A and B). The sulcal area comprises four plates (Figs. 2B and 3A). One of these (Sd) forms a conspicuous flange extending over the median area of the sulcus, partially covering the sulcal area (Fig. 2B). There appears to be a single plate (Ss) beneath this flange (Fig. 2B). Flagella were not preserved in our SEM preparations. In the hypotheca, a series of 5 trapezoid plates of similar size borders the cingulum. A single six-sided antapical plate completes the hypothecal tabulation (Figs. 2E and 3D). The cell surface is mostly smooth. We have never observed a peduncule in either LM or SEM preparations.

Phylogenetic analyses. PCR amplifications of DNA extracts from culture strains and uncultured holobionts led to generation of 35 partial SSU rDNA (~650 bp) and 22 partial LSU rDNA (~675 bp) sequences of dinoflagellate symbionts from spumellarian, collodarian and nassellarian hosts collected in the Mediterranean Sea and in the North and South Pacific oceans (Table 1). For each gene, the vast majority of these sequences were identical (see below) and hence only a subset of 15 SSU rDNA and 10 LSU rDNA sequences, representing a cross-section of host diversity, were included in data sets for phylogenetic reconstructions. Phylogenetic analyses on the SSU and LSU rDNA data sets demonstrated that all of our sequences grouped together in a distinct and highly supported clade

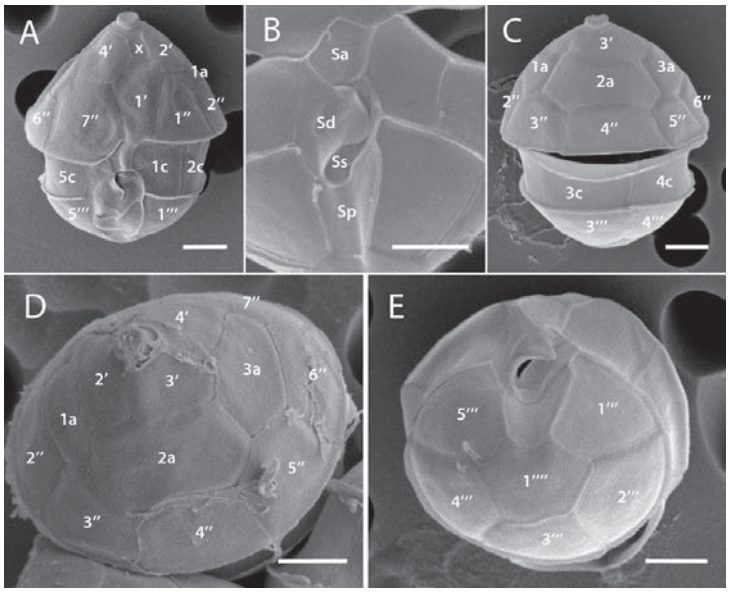

FIG. 2. SEM micrographs of *Brandtodinium nutricula* gen. nov., comb. nov. (enumeration of plates follows the Kofoidian tabulation system). (A) Ventral view of a cell (flagella lost during fixation). (B) Detail of the sulcal region. (C) Dorsal view. (D) Apical view. (E) Antapical view; scale bars = 2 μm.

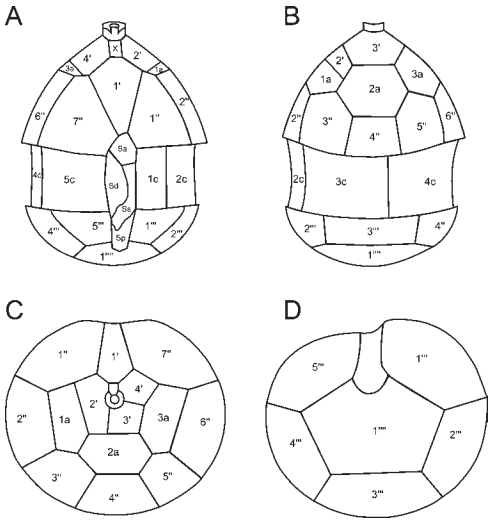

FIG. 3. Schematic representation of plate patterns of *Brandtodinium nutricula* gen. nov., comb. nov. (enumeration of plates follows the Kofoidian tabulation system). (A) Ventral view (generalized). (B) Dorsal view (generalized). (C) Apical view (generalized). (D) Antapical view (generalized).

(hereafter called clade B) within the dinoflagellate order Peridiniales (full ML and Bayesian statistical support; Figs. 4 and 5). In both SSU and LSU rDNA phylogenies, this clade included two distinct subclades, B1 and B2, each containing sequences that are 100% identical irrespective of host taxon and oceanic region. In our SSU rDNA phylogenetic tree (Fig. 4), subclade B1 included the majority of symbiont sequences recovered in this study (including those from five culture strains isolated from *Collozoum* colonies from the Mediterranean Sea and Pacific Ocean), as well as published sequences that correspond to the symbionts of five collodarians and one spumellarian collected in the Atlantic Ocean (Gast and Caron 1996). Subclade B2 contained the sequences generated in the present study of the symbionts of two collodarian holobionts as well as one published sequence (U52357) of the symbiont of the jellyfish *V. velella* (Gast and Caron 1996). In both phylogenetic reconstructions, the monophyletic clade B containing the sequences of polycystine symbionts was phylogenetically distinct from the well-supported clade containing members of the genus *Scrippsiella* (including the holotype species *S. sweeneyae* Loeblich III), but overall the phylogenetic relationships between clades within the Peridiniales were not clearly resolved in our analyses. When sequences of members of the genus *Bysmatrum*, which have a plate tabulation pattern similar to *Scrippsiella*-like peridinaleans (Table 2), were included in phylogenetic analyses, they formed a distinct mono-generic clade which fell on a long branch that altered overall tree topology (Fig. S3 in the Supporting Information). In the SSU rDNA phylogeny (Fig. 4), note that the sequence labeled

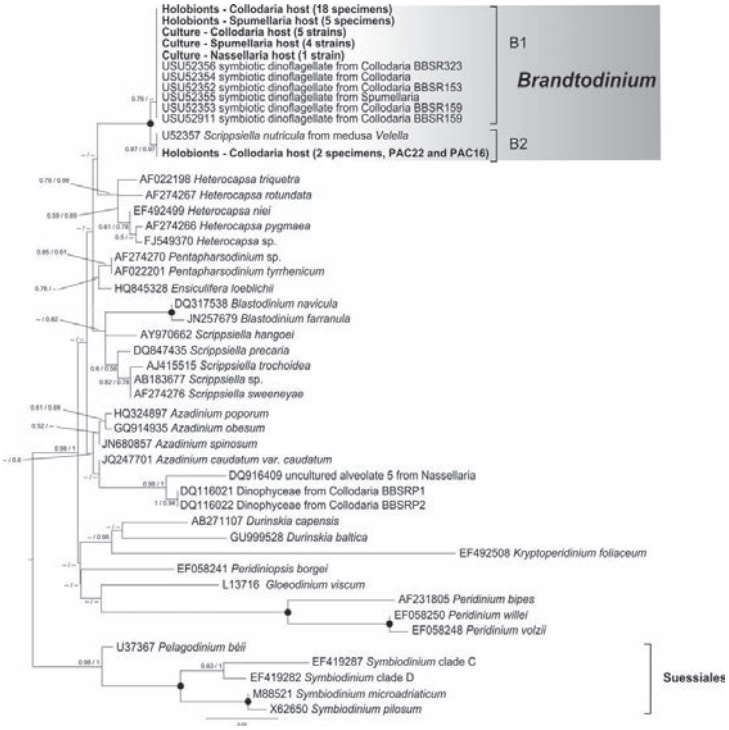

FIG. 4. Small subunit rDNA phylogenetic tree inferred by maximum likelihood (ML) analysis. 652 unambiguously aligned positions were considered from an alignment of 57 sequences, including *Brandtodinium* gen. nov. Sequences obtained in this study are indicated in bold (followed by the type of host from which the sequence was obtained and the number of holobiont specimens or culture strains in parentheses). The tree was rooted with Suessiales (*Symbiodinium* spp. and *Pelagodinium béii*) as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. Numbers on branches are statistical support values for the clusters to the right of them (first: ML bootstrap support values, values under 0.5 are not shown; second: Bayesian post-erior probabilities, values under 0.5 are not shown; black dots at nodes represent a statistical support of 1 for both methods).

"uncultured alveolate from Nasselaria" (DQ916409) and the two sequences labeled "Dinophyceae from Collodaria" (DQ116021 and DQ116022) correspond to nonphotosynthetic dinoflagellate parasites of Radiolaria (Gast 2006).

DISCUSSION

Dinoflagellates that form symbiotic relationships with metazoan or protistan hosts are characterized by complex life cycles, with an alternation of symbiotic and free-living stages with considerable morphological and physiological differentiation between them. Within the host cells, the symbionts are typically coccoid without flagella, and the cingulum and sulcus are no longer apparent (Trench and Blank 1987). In the free-living stage, cells tend to regain their original morphology (Freudenthal 1962, Spero 1987, Siano et al. 2010). Since the taxonomy of dinoflagellates is largely based on comparison of the number, shape, and arrangement of the thecal plates (or amphiesmal vesicles in athecate species) that form the periplast of free-living motile cells, the establishment of clonal cultures from symbionts extracted from their hosts is critical for accurate taxonomic assignation.

The genus *Zooxanthella* was originally created to collectively describe the symbionts of diverse hosts from the Mediterranean Sea, including polycystines, corals, and hydrozoans (Brandt 1881) and *Z. nutricula* was created to describe the symbionts of the collodarian polycystine *Collozoum inerme* (Brandt 1882). The taxonomic history of *Zooxanthella* has subsequently been confusing, with *Z. nutricula* being alternately combined within *Endodinium*, *Amphidinium* Claperède et Lachmann (see review of the nomenclatural history of endosymbiotic dinoflagellates by Blank and Trench 1986) and most recently (albeit technically invalidly) within *Scrippsiella* (Banaszak et al. 1993).

Our observations of the plate tabulation pattern of cultured motile cells of the free-living stage of the dinoflagellate isolated from diverse polycystine hosts clearly show that it is a member of the order Peridiniales (bilateral symmetry, cingulum only slightly displaced, presence of Po and X plates, presence of 3 intercalary plates in the epitheca) and that it should not be classified in the genus *Scrippsiella*, nor in the related genera *Calciodinellum*, *Bysmatrum*, *Pentapharsodinium,* or *Ensiculifera.* All of these latter genera are described as possessing 2 antapical plates, whereas the polycystine symbiont reported here possesses a single antapical plate (Table 2, Figs. 2E and 3D). The presence of a single antapical plate is rare in the order Peridiniales, occurring

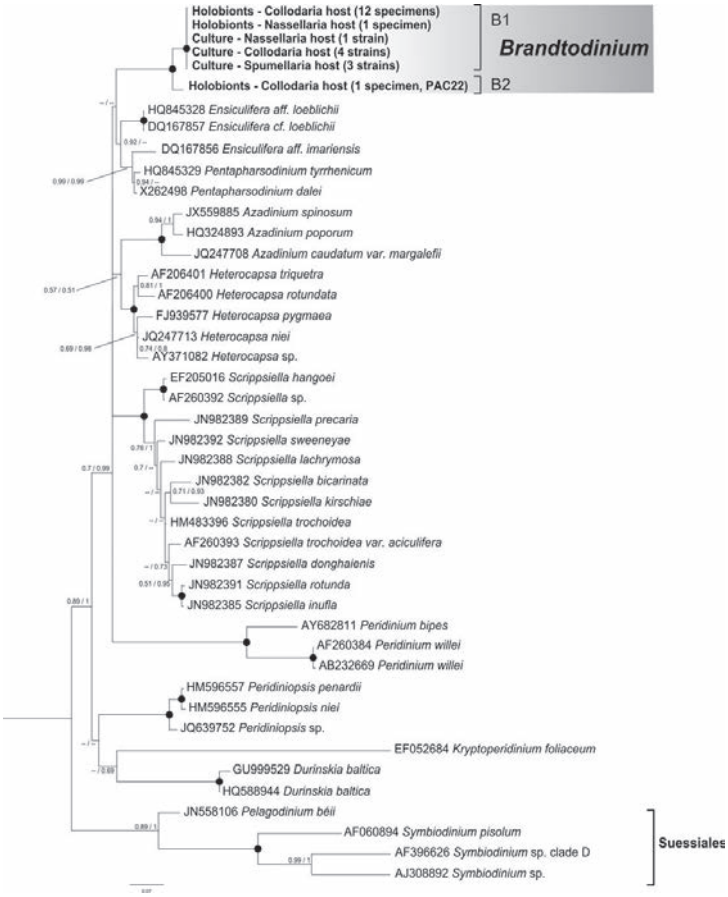

FIG. 5. Large subunit rDNA phylogenetic tree inferred by maximum likelihood (ML) analysis. 675 unambiguously aligned positions were considered from an alignment of 48 sequences, including *Brandtodinium* gen. nov. Sequences obtained in this study are indicated in bold (followed by the type of host from which the sequence was obtained and the number of holobiont specimens or culture strains in parentheses). The tree was rooted with Suessiales (*Symbiodinium* spp. and *Pelagodinium béii*) as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. Numbers on branches are statistical support values for the clusters to the right of them (first: ML bootstrap support values, values under 0.5 are not shown; second: Bayesian posterior probabilities, values under 0.5 are not shown; black dots at nodes represent a statistical support of 1 for both methods).

TABLE 2. Kofoidian plate tabulation of *Brandtodinium* and related genera.

Scrippsiella	Po, X, 4′, 3a, 6-7″, 6c, 4-7s, 5‴, 2⁗
Calciodinellum	Po, X, 4′, 3a, 7″, 6c, 5s, 5‴, 2⁗
Bysmatrum	Po, X, 4′, 3a, 7″, 6c, 4-5s, 5‴, 2⁗
Pentapharsodinium	Po, X, 4′, 3a, 7″, 5c, 4s, 5‴, 2⁗
Ensiculifera	Po, X, 4′, 3a, 7″, 5c, 5s, 5‴, 2⁗
Brandtodinium	Po, X, 4′, 3a, 7″, 5c, 4s, 5‴, 1⁗

notably in a group of heterotrophic genera (*Podolampas* Stein, *Blepharocysta* Ehrenberg, and *Lissodinium* Matzenauer) characterized by the absence of both a cingulum and a depressed sulcus (Gómez et al. 2010) and a group of heterotrophic taxa (*Diplopsalis* Bergh, *Preperidinium* Mangin, *Boreadinium* Dodge et Hermes) characterized by having large lenticular-shaped cells. The radiolarian symbionts are clearly morphologically and ecologically distinct from these other peridinialeans that have a single antapical plate.

The polycystine symbionts also differ from *Scrippsiella* and *Bysmatrum* (but not from *Pentapharsodinium* and *Ensiculifera*) in possessing 5 (rather than 6) cingular plates. The wing-like flange that covers the sulcal area has not been described in any of these related genera. This structure resembles the peduncule cover plate (PC) of heterotrophic dinoflagellates in the peridinialean family Pfiestereaceae Steidinger et Burkholder emend. Litaker. Motile forms of members of the Pfiestereaceae feed myzocytotically by means of a peduncule that emerges close to the flagella and that can attach to microalgal prey or epidermal cells of live fish (e.g., Steidinger et al. 2006). We have not observed a peduncle in the taxon described here, but should it be present, the Sd plate should rather be termed PC and the plate formula would become: Po, X, 4′, 3a, 7″, 5c, 3s, PC, 5‴, 1⁗.

Comparison of morphological characters strongly supports a generic level separation of the

polycystine symbiont reported here from other described Peridiniales taxa, a conclusion that is corroborated by phylogenetic analyses. In both SSU and LSU phylogenies (Figs. 4 and 5), the analyzed polycystine symbionts (including several cultures isolated from *Collozoum* colonies) formed a well-supported clade within the Peridiniales, clearly distinct from *Scrippsiella* and related genera and distant from other dinoflagellate taxa known to form symbiotic relationships such as the suessialeans *Symbiodinium* and *Pelagodinium*.

In light of both morphological and genetic differences from existing genera, this taxon should clearly be classified in a distinct genus. Although *S. nutricula* was previously classified within the genus *Endodinium*, this genus was created to describe the symbiont of *V. velella* from the Mediterranean and there is sufficient doubt as to whether these organisms are actually closely related (see below) to preclude reinstatement of this combination, which in any case should be considered synonymous with *Z. nutricula*. Strict adherence to nomenclatural rules would hence dictate the use of the genus *Zooxanthella* for this species, but we agree with numerous previous authors (e.g., Blank and Trench 1986, Trench and Blank 1987, Banaszak et al. 1993) who have convincingly argued that *Zooxanthella* should be rejected as a confusing name that has been widely applied to divergent taxa. We therefore propose the erection of a new genus, which we name *Brandtodinium* Probert et Siano in reference to Karl Brandt who first described this species (Brandt 1882), and the transfer of *Z. nutricula* to this new genus as *Brandtodinium nutricula* comb. nov. In the absence of a holotype, not provided in the original description of the species, we designate Figure 2, SEM illustrations of plate tabulation of the motile stage of the culture strain RCC3387 of this species, as the neotype for the species.

Although the generic level distinction of *Brandtodinium* from other peridinialeans is obvious, the relationship of this genus to other genera within the Peridiniales is not clear. In terms of overall morphology of the motile stage (e.g., cell size and shape, plate tabulation), *Brandtodinia* has several features in common with members of the Calciodinellaceae Taylor, a family that includes *Scrippsiella*. The Calciodinellaceae, however, are characterized by the production of calcified resting cysts, a feature that we have not observed in *Brandtodinium*. As discussed above, *Brandtodinium* also has certain morphological similarities with members of other groups such as the Pfiestereaceae. An unexpectedly close genetic relationship between *B. nutricula* (as *Z. nutricula*) and a small group of taxa in which photosynthesis takes place by a tertiary endosymbiont derived from a diatom (Horiguchi and Pienaar 1994), the "dinotoms" (Imanian et al. 2011), was recently reported (Gottschling and McLean 2013). These investigators employed a "maximal taxon sample" approach by inferring relationships based on a concatenated SSU, LSU and internal transcribed spacer (ITS) rDNA sequence alignment irrespective of whether all of these sequences were available for the taxa included (i.e., an alignment with significant gaps). Our individual SSU and LSU phylogenies do not recover this relationship. This study provides strong evidence from two highly conserved phylogenetic markers (SSU and LSU rDNA) to support the conclusion from our observations of the morphology of free-living cells that *Brandtodinium* is a taxonomically distinct genus within the Peridiniales. We chose not to employ an approach comparable to that of Gottschling and McLean (2013) because in-depth assessment of evolutionary and phylogenetic relationships between *Brandtodinium* and other members of the order Peridiniales goes beyond the scope of our research. We nevertheless provide evidence that *Brandtodinium* is distinct from the dinotom genera (*Durinskia* Carty et Cox, *Galeidinium* Tamura et Horiguchi, *Kryptoperidinium* Lindemann, and some species currently assigned to *Peridiniopsis* Lemmermann or *Peridinium* Ehrenberg) on the basis of morphological criteria, notably because dinotom genera all have two antapical plates, whereas *B. nutricula* possesses a single antapical plate, but also because the characteristic highly visible eyespot of dinotoms is absent in *B. nutricula*.

Banaszak et al. (1993) described the dinoflagellate symbiont of the jellyfish *V. velella* from the Pacific as *S. velellae* and also (albeit invalidly) transferred *Endodinium* (=*Zooxanthella*) *chattonii*, the symbiont of Mediterranean *V. velella*, to *Scrippsiella*, as *S. chattonii*. These authors gave the thecal plate formula for *S. velellae* as pp (=Po, X), 4′, 3a, 7″, 5c, 3s, 5‴, 2⁗, which corresponds neither to that of *Scrippsiella* nor to that of *Brandtodinium* (Table 2). The spine-like protuberance on the first cingular plate illustrated in figure 11 (p. 520) of Banaszak et al. (1993) is a characteristic feature of the genus *Ensiculifera*, to which we believe this species should have been assigned. However, the SEM images illustrated in Banaszak et al. (1993) do not permit verification of whether this organism really has 3 sulcal plates (as stated in the description), rather than 5, as diagnostic for members of the genus *Ensiculifera*. It could also be inferred that *S. chattonii*, the symbiont of Mediterranean *V. velella*, might also be transferred to *Ensiculifera*, but unfortunately no morphological data has ever been provided for the free-living stage of this taxon. It is noteworthy that the only existing sequence (SSU rDNA) of a symbiont of *V. velella* (from the Sargasso Sea, Atlantic Ocean) produced by Gast and Caron (1996) falls within our *Brandtodinium* clade, in the subclade B2 composed of three identical sequences, two of which we generated from Pacific polycystine holobionts. This subclade is distinct from the subclade B1 formed by the group of identical sequences from all of our Pacific (South and North) and Mediterranean culture strains of

B. nutricula isolated from polycystines, from several Pacific polycystine holobionts that we sequenced, and from the Sargasso Sea polycystine symbionts sequenced by Gast and Caron (1996). Gast and Caron (1996) did not observe the morphology of the dinoflagellate symbionts of Sargasso Sea *V. velella* that they sequenced, but we predict that they would have plate tabulation consistent with our description of *Brandtodinium*. If this was the case, it would mean that *V. velella* is capable of forming symbiotic associations with different dinoflagellate genera (*Brandtodinium* and *Scrippsiella* (or *Ensiculifera*)), possibly with a biogeographical pattern (*Brandtodinium* in the Atlantic and possibly Mediterranean, *Scrippsiella* (or *Ensiculifera*) in the Pacific). The capacity of hosts to form associations with different symbionts has already been observed for other pelagic organisms (Siano et al. 2010, Decelle et al. 2012b). A comparison of genetic sequences from morphologically characterized cultured *V. velella* symbionts from the Pacific Ocean, Sargasso Sea, and Mediterranean Sea could be helpful in establishing the validity of historical descriptions of these symbionts and their relationship with *B. nutricula*.

Brandtodinium has been found (in this and previous studies) in association with diverse polycystine radiolarian hosts from the North and South Pacific Ocean, Sargasso Sea, and Mediterranean Sea. In light of the abundance of symbiotic polycystines in the world ocean, *Brandtodinium* likely plays a key ecological role in primary and secondary production at a global scale. Putting aside associations with parasitic alveolates (Gast 2006, Bråte et al. 2012) that can be considered as a form of symbiosis, all Collodaria investigated so far harbor only *Brandtodinium* species as symbionts. At present, *Brandtodinium* is the only symbiont identified for Nassellaria, but information for this radiolarian group remains extremely scarce. *Brandtodinium* has now been found in association with numerous spumellarian hosts, but unlike the other polycystine lineages, other types of (non dinoflagellate) microalgal and cyanobacterial symbionts have also been reported for this group (Anderson 1983, Gast and Caron 2001, Yuasa et al. 2005). With *Brandtodinium* also probably found in symbiosis with jellyfish, it is clear that *Brandtodinium*, like the suessialean dinoflagellates *Pelagodinium* and *Symbiodinium*, is a generalist symbiont. In this context it is interesting to note that the known genetic diversity (in terms of SSU and LSU rDNA sequences) of *Brandtodinium* and *Pelagodinium*, both of which form symbiotic relationships with planktonic hosts, is relatively low (2 clades described within each of these genera) compared to that of *Symbiodinium* (9 divergent clades and multiple subclades, Stat et al. 2008, Pochon and Gates 2010) that is predominately found in association with benthic host organisms. This apparent trend might be explained by the relatively low number of studies on symbiosis in the pelagic realm, but might also be real and reflect inherent differences between life and symbiotic processes in planktonic and benthic ecosystems (Decelle 2013).

Taxonomic appendix. ***Brandtodinium*** Probert et Siano **gen. nov.**

Diagnosis: Photosynthetic dinoflagellate. Motile cells covered by 6 series of thecal plates: 3 in the epitheca, 2 in the hypotheca (including single antapical plate), and 1 in the cingulum. One transverse and one longitudinal flagellum. Large nucleus located in central part of cell. One or two peripheral chloroplasts, golden-yellow in color. One or two large circular pyrenoids.

Type species: *Brandtodinium nutricula* (Brandt) Probert et Siano *comb. nov.*

Etymology: the genus name for this dinoflagellate (= *dinos*) derives from Karl Brandt who first described *Zooxanthella* in 1882.

Brandtodinium nutricula (Brandt) Probert et Siano **comb. nov.**

Basionym: *Zooxanthella nutricula* Brandt in Brandt (1882) *Archiv für Anatomie und Physiologie Leipzig 1882*: 140.

Synonyms: *Endodinium nutricula* (Brandt) Hollande et Carré in Hollande and Carré (1974); *S. nutricula* (Brandt) Banaszak, Iglesias-Prieto et Trench in Banaszak et al. (1993).

Neotype: Fig. 2 in this publication.

Diagnosis: Plate tabulation: Po, X, 4′, 3a, 7″, 5c, 4s, 5‴, 1⁗. Epitheca larger than hypotheca. Epitheca convex conical with well-pronounced apical horn. Hypotheca rounded. Wide and shallow cingulum located in the median portion of the cell, displaced by a small fraction of its own width. Sulcal area with 4 plates, one of which forms a wing-like flange over the median part of the sulcus. Single antapical plate. Cells on average 13.1 μm in length by 10.4 μm in width. Symbiont of polycystine radiolarians.

Type locality: Bay of Villefranche-sur Mer (France), Western Mediterranean Sea

Authentic culture strain: RCC3387 in the RCC:

Following the production process of this manuscript, the authors will submit a formal proposal to the ICN to reject the genus *Zooxanthella*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. LM images of host cells from which uncultured symbiont (holobiont) sequences were retrieved.

Figure S2. LM images of host cells from which cultures were isolated.

Figure S3. SSU rDNA phylogenetic tree inferred by ML analysis. 652 unambiguously aligned positions were considered from an alignment of 59 sequences, including *Bysmatrum*. The tree was rooted with Suessiales (*Symbiodinium* spp. and *Pelagodinium béii*) as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. Numbers on branches are statistical support values for the clusters to the right of them (first: ML bootstrap support values, values under 0.5 are not shown; second: Bayesian posterior probabilities, values under 0.5 are not shown; black dots at nodes represent a statistical support of 1 for both methods).

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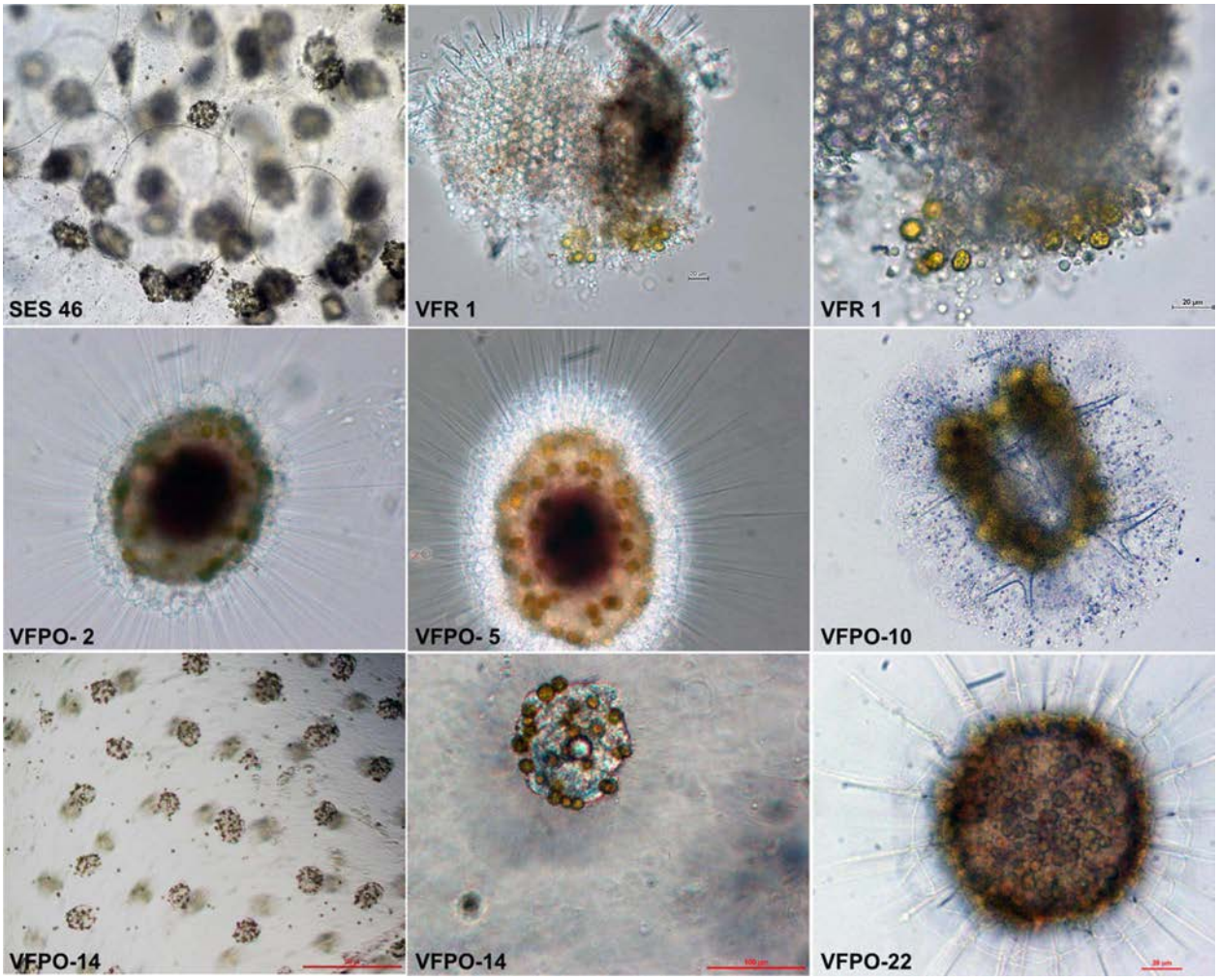

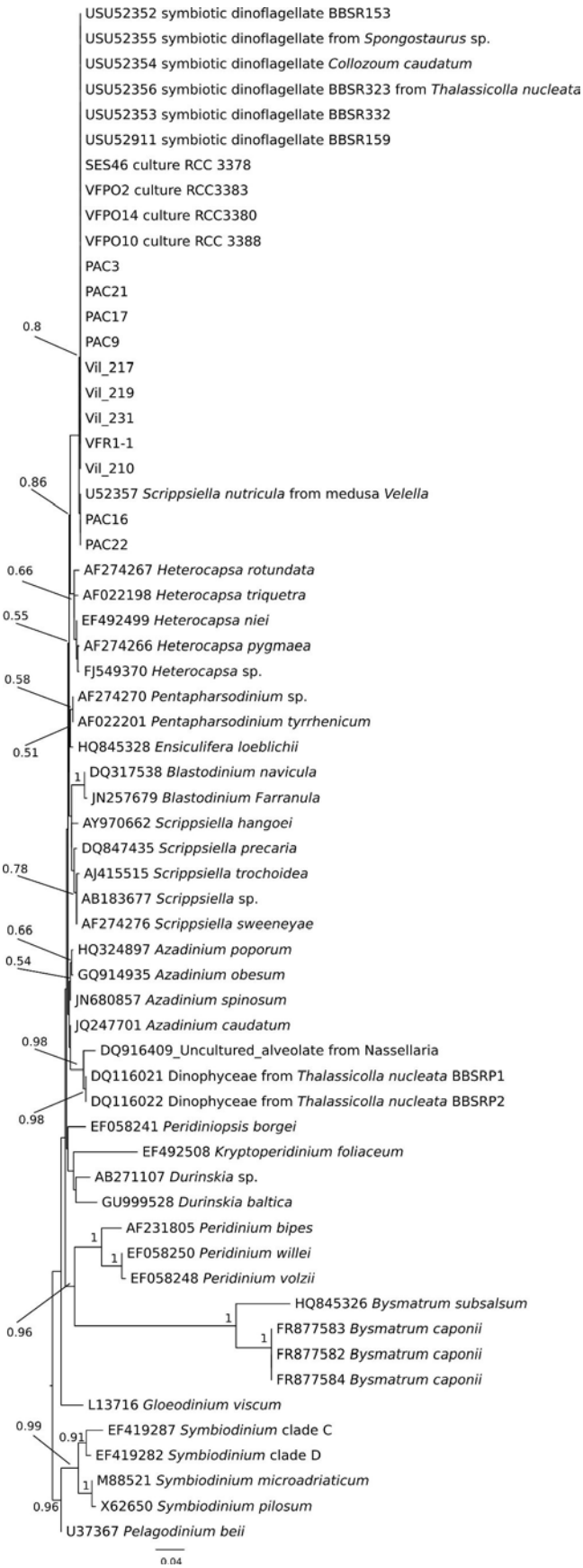

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Abstract

Collodaria (Radiolaria) are unicellular marine eukaryotes (protists) belonging to the super-group Rhizaria. Collodarian species contribute to planktonic communities as large solitary cells or can form large gelatinous colonies. They are heterotrophic organisms feeding on other plankton, which also systematically harbour intracellular symbiotic microalgae. Recent environmental molecular diversity surveys demonstrated their important contribution to planktonic communities and their worldwide occurrence in the global ocean. However, knowledge on their diversity, biogeography and ecology is paradoxically very poor.

In the first part of this thesis I performed detailed morphological analyses (electron and optical microscopy) combined with a molecular phylogeny based on the 18S and 28S rRNA genes, sequencing for a total of 75 distinct colonial and solitary specimens. Ultimately, this work led to the revision of the Collodaria classification and to the construction of a robust morpho-molecular reference database. Then, this morpho-molecular framework allowed the exploration of Collodaria biodiversity through a metabarcoding approach across samples collected in the global ocean during the *Tara* Ocean expedition. The cosmopolitan distribution of the different collodarian taxa in the surface oceans revealed a higher biodiversity in the vast oligotrophic inter-tropical open oceans. Collosphaeridae were predominantly found in the open oceans while the Sphaerozoidae were the dominant family in the less diverse coastal regions. The newly defined Collophidiidae were rarely encountered in the photic zones at all latitudes, suggesting that they inhabit a different ecological niche. Finally, I also used the *in situ* imaging system Underwater Vision Profiler (UVP5) to quantitatively explore the abundances and biomasses of collodarian and rhizarian in the global ocean. This approach revealed that the Rhizaria were a major component of the meso- and macro-plankton, constituting up to 4.5% of the global carbon standing stock in the upper 200 m of the world oceans. More specifically, Collodaria were the most important rhizarian groups in the first 100 m of the oceans, and their distribution suggested that photosymbiosis might be an important factor explaining their success in oligotrophic regions where they are particularly abundant. Besides the improvement of our knowledge on the diversity, biogeography and ecology of Collodaria in the global ocean, this thesis highlights the relevance to combine and/or use alternative sampling and analytical procedures such as high-throughput sequencing and *in situ* imaging technologies to study marine protists in their environment.

Keywords: Collodaria; Radiolaria; Rhizaria; Global ocean; *In situ* imaging; High-throughput sequencing; Metabarcoding; Integrative taxonomy; Molecular phylogeny; Zooplankton.